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EAST HELENA, MONTANA

CHILD LEAD STUDY

SUMMER 1983

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Participating Agencies

Lewis and Clark County Health Department
Montana Department of Health and Environmental Sciences
Center for Environmental Health, Centers for Disease Control,
Public Health Service, U.S. Department of Health and Human Services,
Atlanta, GA 30333
U.S. Environmental Protection Agency

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Final Report - July, 1986
Corrected Copy

JUL 6 1991

APR 8 - 1992

NOV - 9 1994

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

Executive Summary

In 1983, an integrated epidemiologic study was conducted in the Helena Valley of Montana to assess children's blood lead levels and the relationship of these levels to the levels of lead in different environmental media. Blood samples, environmental samples, and questionnaire data were collected for 396 children living at various distances from an operating primary lead smelter in East Helena, Montana. Analyses of these samples showed that children who lived close to the smelter had higher blood lead levels (13 micrograms per deciliter (ug/dl)) than children who lived farther away (6 ug/dl), although both groups had similar erythrocyte protoporphyrin levels. Multiple regressions identified dust lead levels, air lead levels, residence near the smelter, and having a household member who smokes as major contributors to the dependent variable of blood lead. One child living near the smelter had lead toxicity, i.e., a blood lead level ≥ 25 ug/dl and an erythrocyte protoporphyrin level ≥ 35 ug/dl. The blood lead levels of all other children tested, however, showed no cause for public health concern. No health risk assessment was made from the study for any substance other than lead.

Two substudies were conducted within the framework of the above study. One consisted of measuring heavy metals in hair and urine samples for a small number of children. These analyses showed that children who lived near the smelter had hair lead levels greater than children who lived farther away, while both groups had similar mean urinary lead levels. The other substudy consisted of estimating toddlers' daily soil ingestion by measuring the amounts of aluminum, silicon, and titanium in their stools. Soil ingestion estimates were calculated for 59 children aged 1-3 years. Estimated daily soil ingestions based on aluminum and silicon were 121 and 184 mg/day, respectively; the estimate based on titanium was about 10 times higher--1,834 mg/day. Details on this substudy are in Appendix 23.

An earlier version of this report underwent peer-review. This version incorporates additional information and analyses recommended by that group.

Agency Letters Regarding This Report

DEPARTMENT OF HEALTH AND ENVIRONMENTAL SCIENCES



TED SCHWINDEN, GOVERNOR

COGSWELL BUILDING

STATE OF MONTANA

HELENA, MONTANA 59620

December 13, 1985

Vernon N. Houk, M.D.
Assistant Surgeon General
Director
Center for Environmental Health
Centers for Disease Control
Atlanta, GA 30333

Dear Dr. Houk :

The East Helena, Montana Child Lead Study Report, Summer 1983, which was jointly done by Montana's Department of Health and Environmental Sciences (DHES), the Environmental Protection Agency (EPA), and Centers for Disease Control (CDC) of Atlanta, satisfies a long-standing need by East Helena families for information as to health risks associated with living near an operating primary lead smelter. This comprehensive study, designed by DHES and CDC, is presented here in what we consider a very readable, well done report.

Three major sources of lead contribute to blood-lead levels in East Helena children ages one-to-six years: house dusts, soils, and airborne particulates.

The study, however, is not complete without recommendations for follow-up actions. These statements are grounded, not only by results from this study, but also by DHES personnel who: 1) have responded to complaints from residents in East Helena, and 2) who have pursued clean-up measures for many years. For people to safely live near the smelter the following are essential:

1. Ambient air-lead concentrations must be decreased to comply with the national and Montana ambient air standard of 1.5 ug lead/m³ of air, quarterly average;
2. Residents in and around East Helena should wash and limit their consumption of local broad-leafed garden vegetables;
3. Children should be strongly discouraged from eating snow which accumulates high concentrations of toxic heavy metals;

Vernon N. Houk , M.D.
Page Two
December 13, 1985

4. Young children put things other than food into their mouths as part of their normal playing activity. Because of this, parents should be instructed about the possibility of lead exposure from contaminated soils and house dusts and what precautions parents can take to minimize this exposure.
5. Area physicians should continue their awareness for potential cases of lead toxicity and report such cases to DHES.

Sincerely,



A. David Maughan, M.A.
Project Officer



John J. Drynan, M.D.
Director

ADM:JJD:sqj
cc: East Helena File

Centers for Disease Control
Atlanta GA 30333

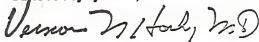
December 31, 1985

John J. Drynan, M.D.
Director
Montana Department of Health
and Environmental Sciences
Cogswell Building
Helena, Montana 59620

Dear Dr. Drynan:

Thank you for your letter of December 13, 1985, in which you listed the recommendations which the Montana Department of Health and Environmental Sciences has made regarding lead exposures in East Helena, Montana. The Center for Environmental Health agrees that these recommendations are sound.

Sincerely yours,



Vernon N. Houk, M.D.
Assistant Surgeon General
Director
Center for Environmental Health

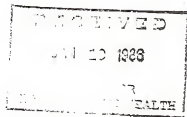




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JAN 6 1986

Vernon N. Houk, M.D.
Assistant Surgeon General
Director, Center for Environmental
Health
Centers for Disease Control
Atlanta, Georgia 30333



Dear Dr. Houk:

We are pleased to learn that your agency is soon to release the final report entitled "The East Helena, Montana Child Lead Study Report, Summer 1983." This report will be most important to the completion of EPA's "Superfund" investigations at the East Helena site.

We have recently received a copy of a letter addressed to you and written by Mr. David Maughan, Project Officer, and Dr. John Drynan, Director, Montana Department of Health and Environmental Sciences (MDHES). This letter contains a list of five recommendations that the MDHES believes should be implemented at East Helena to lessen the exposure of residents to elevated lead levels in area soils, household dust, and ambient air.

Mr. Gene Taylor, EPA Project Officer, Mr. Maughan, and Dr. Rebecca Schilling, CDC Project Officer, have previously agreed that the MDHES letter should be attached to the CDC's final report. It was also agreed that the EPA should furnish the CDC a letter expressing our position on the State's recommendations. The EPA position is as follows:

We strongly agree that ambient air-lead concentrations at East Helena must be brought into compliance with State of Montana and Federal standards (Refer to Recommendation No. 1). The Montana Department of Health and Environmental Sciences is currently negotiating with ASARCO on ways to bring the smelter operation into compliance with the ambient lead standard. This must proceed in a timely manner.

We also believe the MDHES recommendations concerning ingestion of broad-leaf vegetables, ingestion of snow, the education of parents concerning exposure pathways, and the need for local physician awareness of potential lead problems (Recommendations 2-5) also have merit. These recommendations are based on the MDHES' extensive understanding of the East Helena situation and appear to EPA to be a prudent course of action to be followed by area residents.

We should point out that all of the State's recommendations will be further considered during EPA's evaluations under the Superfund effort. These evaluations will include examining a number of possible solutions to correct any adverse health or environmental impacts that are occurring in the study area due to elevated levels of metals, including lead. CERCLA also provides that we coordinate these efforts with those being undertaken under authority of the Clean Air Act.

We would like to thank you and all of your excellent staff at the CDC for completion of the East Helena study. We believe the study to be a most important contribution to understanding the East Helena situation and to our own Superfund investigations.

Sincerely,

A handwritten signature in dark ink, appearing to read "John F. Wardell". The signature is fluid and cursive, with the first name "John" being the most prominent.

John F. Wardell, Director
Montana Office

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1.0 History and Introduction

The East Helena lead smelter was built in 1898 by the Helena and Livingston Smelting and Reduction Company with assistance from citizens of nearby Helena. In 1899, plant ownership changed to the American Smelting and Refining Company (ASARCO). At present, the smelter processes domestic and foreign ores and concentrates from some western States, Canada, South America, and Australia.

In 1927, the Anaconda Company built a zinc-recovery plant adjacent to the ASARCO lead smelter. In 1955, American Chemet Corporation constructed a paint pigment plant that used zinc oxide from the Anaconda plant. In 1972, ASARCO purchased the zinc-recovery plant from Anaconda. In 1982, ASARCO closed the zinc operation because of a depressed market. With this closure, the ASARCO work-force decreased from 360 to about 320 employees. The American Chemet paint pigment plant has continued to operate and employs about 40 people.

In 1983, the lead smelter processed some 300,000 tons of material and produced about 60,000 to 100,000 tons of lead bullion and about 35,000 tons of zinc oxide.

The smelter is in an agricultural area of the Helena Valley of west central Montana. The valley, about 25 miles from north to south and 35 miles east to west, lies between the Big Belt Mountains to the north and east and the Continental Divide to the west. Valley elevation in East Helena is about 3,900 feet; adjacent mountains rise an additional 3,000 feet. Seasonal weather changes in East Helena are typical for north latitude mountains. Summer highs of 30 degrees centigrade (°C) and winter lows of -30°C are typical. Northern and eastern portions of the valley are semiarid, receiving 9-10 inches of precipitation annually. Western areas near the Continental Divide may receive up to 30 inches of precipitation, mostly in the form of snow. The predominant direction of wind flow is from west to east. This is modified by diurnal upslope and downslope surface winds on the surrounding mountain ranges. The mountain ranges tend to protect the valley from major winds and thereby create a pocket effect. The resulting inversions trap cold air near the ground.

Particulate and gaseous emissions from the smelter and from the smaller paint pigment factory have contaminated air, soil, and surface water in the East Helena area, as shown by the results of an environmental study conducted jointly by the Montana State Department of Health and the U.S. Environmental Protection Agency in 1969 and 1970¹. At that time, air emissions of arsenic, cadmium, and lead from both plants had contaminated area soils and vegetables to levels identified as toxic to grazing

livestock. Public health interventions based on these findings included recommending that (a) low-grazing farm animals such as horses and sheep should not graze in fields near East Helena and (b) people living in the East Helena area should wash locally grown vegetables to remove surface contamination before eating them.

In February of 1975, the Montana State Department of Health and the Centers for Disease Control (CDC) conducted a study of children's exposures to smelter-associated lead in East Helena. Blood samples were collected from 90 children aged 18 months to 10 years. CDC analysis of these samples showed a mean blood lead level of 28.0 ± 8.8 micrograms per deciliter (ug/dl); individual values ranged between 15-67 ug/dl. Blood lead levels of 9 children exceeded 40 ug/dl. Blood lead levels of 31 children exceeded 30 ug/dl. At present, children with blood lead levels of 25 ug/dl or higher are considered to have elevated blood lead levels². The ground cover of 12-14 inches of snow that was present during the sampling probably reduced the children's exposures to lead-contaminated soil. The child who had the highest blood lead level measured, 67 ug/dl, was reported to have eaten snow.

In 1978 and 1979, the Montana State Department of Health and Environmental Sciences (MDHES) investigated reports of several cattle deaths in herds pastured near the smelter during the winter. As part of this investigation, MDHES analyzed snow cores collected from around the smelter and from comparison areas for lead, zinc, arsenic, and cadmium. From these measurements, MDHES estimated that about 14 tons of lead, 13 tons of zinc, 1.16 tons of arsenic, and 0.16 tons of cadmium were deposited annually on East Helena and nearby areas³. MDHES estimated that, in 1978-79, if a child ate a portion of East Helena snow equivalent to one 12-ounce soft drink, he or she would ingest 5-10 mg lead, 5-10 mg zinc, 0.5-1.2 mg arsenic, and 0.05-0.10 mg cadmium. MDHES concluded that the most likely cause of the cattle deaths was ingestion of high concentrations of heavy metals in small pools of melted snow.

Abandoned mining operations in the mountains south of East Helena have contaminated the headwaters of the Prickly Pear Creek with acid water and with heavy metals, and further downstream the creek flows past the base of the ASARCO slag pile. Nevertheless, the water in this creek is suitable for drinking, food processing, bathing, and swimming uses within East Helena. North of the city, the creek receives Helena and East Helena discharge from municipal wastewater treatment facilities, and the water quality becomes suitable only for crop irrigation and industrial needs.⁴ Tests done before 1980 indicate that levels of heavy metals in East Helena groundwater are acceptable.

In 1980, ASARCO funded a study to determine the stack height needed to prevent the deposition of sulfur dioxide emissions in East Helena⁵. As part of this study, vertical trajectories of constant-volume balloons traveling over the smelter stacks were compared with those traveling distant from the smelter stacks. The comparison confirmed that light winds traveling in a northerly direction over the smelter complex are first thrust upward by the heat from smelter blast furnaces and then, after passing over the smelter complex, downward as they cool. The Helena Valley commonly has light northerly winds as a result of early morning downslope breezes from the adjacent mountain ranges. Thus, East Helena, which lies north of the smelter complex, is likely to receive emission fallout.

In 1981-82, MDHES funded a study of emission sources of ambient air lead in East Helena. This study showed four major sources: (1) the ASARCO blast furnace operation, (2) the ASARCO ore handling and storage activities, (3) the ASARCO zinc operations, and (4) highways and streets adjacent to the smelting complex⁶. MDHES concluded that the roadway lead emissions largely resulted from industrial emission fallout with dust resuspensions due to traffic flow. On the basis of these findings, ASARCO began dust-control measures, such as street sweeping programs, revegetation of the smelter property perimeter, paving of smelter property, and new methods for storing ores and concentrates. Ambient air lead concentrations, however, remained the same.

In 1982, MDHES collected window-sill dust for heavy metal analysis from eight households on Pacific Avenue, the closest city street parallel to the ASARCO slag pile. Analyses showed high mean levels of lead (62,949 parts per million (ppm)), copper (245,546 ppm), and arsenic (2,295 ppm)⁷.

The population of East Helena is 1,650. An additional 1,500 residents live outside the city in neighborhoods to the north and east. About one-third of the smelter property lies within the city limits in close proximity to these residential neighborhoods. The number of children living in these neighborhoods had been increasing during 1981-82 as young families moved in. The student population of the elementary and middle schools was expected to continue to grow during the rest of the decade.

On the basis of the above information, in September 1982, MDHES requested assistance from the Center for Environmental Health, CDC, in evaluating residential exposures to smelter-associated lead and other heavy metals in neighborhoods near the smelter.

In response to this request, the study described in this report was conducted in the summer of 1983. This study was funded through (1) an interagency agreement between the U.S. Environmental Protection Agency (EPA) and the Department of Health and Human Services (DHHS)/CDC and (2) a cooperative agreement between DHHS/CDC and MDHES. Funding for DHHS/CDC came from the Agency for Toxic Substances and Disease Registry using monies from the trust fund established by the Comprehensive Environmental Response, Compensation, and Liability Act.

Agency responsibilities under these agreements were:

EPA Responsibilities:

1. To provide monies to CDC to support a study of residential exposures to lead and other heavy metals in neighborhoods near the East Helena smelter;
2. To provide MDHES with technical assistance and chemical analyses for quality assurance purposes; and
3. To review all reports of study progress and findings.

CDC Responsibilities:

1. To provide on-site training and supervision for biological sampling;
2. To analyze special biological samples;
3. To edit data tapes and provide statistical analyses;
4. To review study progress; and
5. To assist MDHES with report development.

MDHES Responsibilities:

1. To provide overall coordination of the study;
2. To conduct all biologic and environmental sampling and analyses, with the exception of the analyses of hair samples for adults and children and the analyses of other adult biologic samples;
3. To prepare quarterly reports on study progress;
4. To perform statistical analyses; and
5. To develop a final study report.

The study supported by these agreements had four objectives: (1) to measure blood lead levels in children, the population likely to be at greatest risk of being exposed to lead and of having adverse health effects from lead exposure; (2) to determine

whether these blood lead levels were associated with the amount of lead in the various environmental media of the children's usual surroundings; (3) to assess the exposures of both children and adults to other heavy metals; and (4) to estimate the amount of dirt ingested by children during normal play.

The following report addresses the procedures and results of these objectives.

2.0 Materials and Methods

2.1 Selection of Sampling Period

Biologic and environmental samples were collected during August and September 1983 to obtain blood levels that reflect summer exposures. Children's exposures to smelter-associated lead are likely to be highest during the summer when (1) warm weather permits outside play, (2) soil conditions are dusty, (3) open windows increase the amount of dust entering houses, and (4) more locally grown vegetables and fruits may be eaten.

2.2 Operations Center

The operations center for the data collection phase of this study was in the offices of the MDHES Air Quality Bureau in the Cogswell Building, Helena, Montana. MDHES staffed the operations center with supervisory personnel retained by subcontract to the Lewis and Clark County Health Department.

2.3 Designation of Study Areas

Three study areas were designated according to their distances from the smelter in East Helena. These areas and their respective distances were:

Area 1: Within 1 mile of the smelter

Area 2: 1-2.25 miles from the smelter

Area 3: More than 5 miles from the smelter (see Figure 1)

Figure 1 shows the relative locations of the three study areas.

2.4 Census

A census was conducted to identify all children ages 1 through 5 years within Areas 1, 2, and 3. Workers familiar with the 1980 U.S. Census made door-to-door contacts within the study areas to explain the need for the study and to administer questionnaires. Newspaper and radio announcements and letters to the residents preceded the census workers (Appendices 1-2). The census

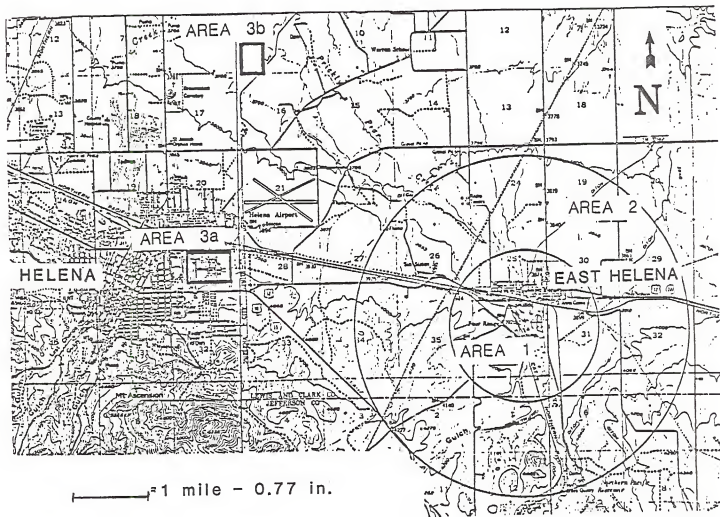
questionnaire (Appendix 3) was designed to locate families with children and with gardens and to determine the length of residency and to obtain a partial adult employment history. Eligible households were defined as those with children ages 1 through 5 who had lived in the particular study area for 3 months or more.

Census workers were instructed to make, if necessary, three visits to each residence in an attempt to interview the family at that address before interviewing the nearest neighbors as a secondary source. Census workers updated local maps for residence identification (Appendix 4) and prepared a file system of eligible households at the operations center consisting of a separate file for each eligible household.

Census findings in Area 3a (Figure 1) showed fewer children and adults than we had predicted by using the 1980 U.S. Census and aerial residential counts. To obtain sufficient children for the study, census workers canvassed a subdivision north of Helena (3b) which was similar in age, race, and socioeconomic characteristics to Area 3a.

Figure 1

1983 Study Areas: East Helena and Helena, Montana
(Area 1 and Area 2 boundaries not drawn to scale)



2.5 Blood Sample Collection Strategy

The plans for using temporary clinical facilities in East Helena for blood sample collection, as described in the study protocol, were abandoned for the following reasons:

- (1) Collection of environmental samples from each child's home would be essential to both (a) evaluating his or her blood lead level and (b) meeting the study objective of determining whether blood lead levels of children in the community are associated with environmental lead levels.
- (2) Arranging for simultaneous collection of blood samples and environmental samples would increase the likelihood of obtaining both types of samples for each child.
- (3) The benefits of obtaining both types of samples for each child would justify having medical technologists visit individual homes.

2.6 Interviewing and Household Sampling

Five teams, each comprised of an interviewer, a medical technologist, and an environmental technician, were trained by MDHES and CDC staff to understand and perform the following activities:

- (1) Obtain participant consent using a standard form (Appendix 5).
- (2) Administer questionnaires precoded for entry onto computer tape (Appendix 6).
- (3) Obtain blood samples from all children ages 1 through 5 in eligible households (Appendix 7), using lead-free vacutainer collection tubes manufactured by Becton-Dickinson. Collect hair and urine samples from a limited number of children in Areas 1 and 2 (Appendices 8-9). Collect handwash samples from all eligible children in the study areas (Appendix 10).
- (4) Obtain environmental samples or readings: yard soil samples at 1- and 3-inch depths (Appendix 11); household vacuum bag dust samples from all homes possible and special vacuum filter samples from 50 randomly selected homes in the three study areas (Appendix 12); x-ray fluorescence readings of the lead content of interior and exterior household paints (Appendix 13); kitchen linoleum swab dust samples (Appendix 14); garden vegetable and garden soil samples (Appendix 15); and summarize all soil and dust samples collected on the appropriate form (Appendix 16).

- (5) Assign a rating for household cleanliness according to a four-point scale (Appendix 16).
- (6) Record the approximate percentage of grass cover in the front yard, backyard, and play area (Appendix 16).

Team members practiced conducting interviews and collecting samples at the homes of MDHES staff before visiting study participants.

Introductory letters explaining the need for a comparison group were mailed to eligible households in Area III before study teams visited them (Appendix 17). At first, teams received lists of homes to be visited from operations center personnel. Frequently, however, the teams found many families not to be at home. Consequently, operations center personnel arranged appointments by telephone for home visits. With this method, teams completed visits to 8-10 homes per day.

Teams obtained forms, supplies, equipment, and appointments on a daily basis from operations center personnel. Field teams returned to the operations center upon completing the home visits each day to submit questionnaires, data sheets, and biologic and environmental samples to a clerk for purposes of chain-of-custody (see section 2.10).

2.7 Ambient Air Sampling

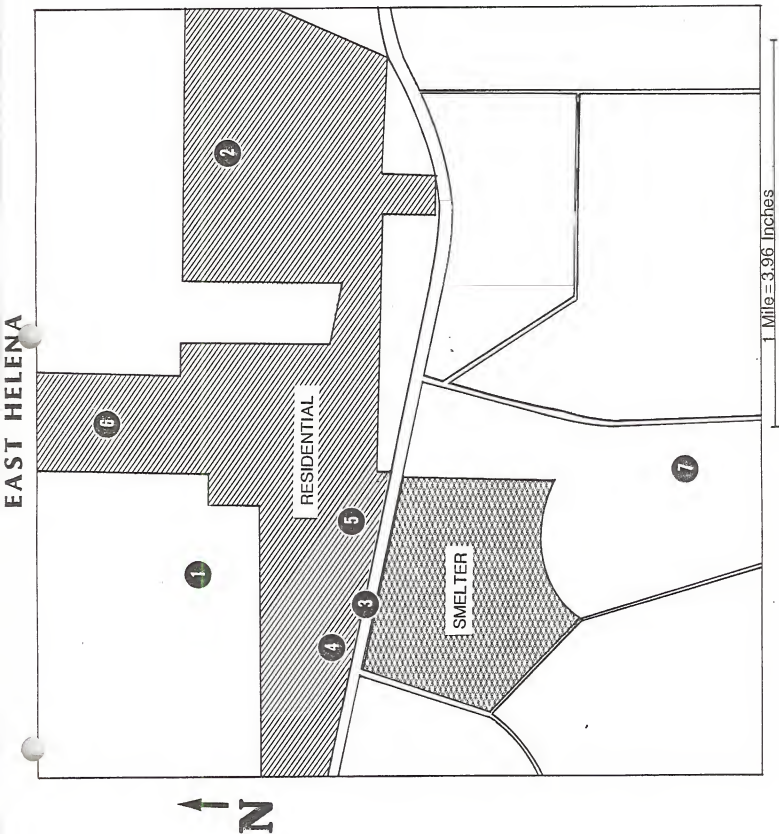
Before and during the field portion of this study, MDHES collected airborne particulates from eight monitoring sites shown in Figure 2. These sites were sampled to characterize airborne particulates in the three study areas. Monitoring sites in Area 1 included the Dartman, Firehall, Hadfield, and Hastie stations. Monitoring sites in Area 2 included the Dudley, Schneider, and South stations. The Townsend monitoring site was in Area 3. The Department had already established several of these stations as a part of the ongoing air quality effort. The locations of the monitors were chosen on the basis of estimated maximum impact (Dartman, Firehall, Hadfield, and Hastie), population, and geography. The Schneider station, for example, indicates the ambient concentration to the north of the smelter in Area 2. The Dudley site represents the East Gate subdivision in Area 2. The South site is also in Area 2 but in the opposite direction of the other Area 2 monitors.

Only one site was chosen for Area 3 (Townsend), since previous air monitoring showed low lead concentrations in the Helena area. Each station consisted of a high-volume air sampler operated on a 1-day-in-3 frequency. The high-volume sampler was chosen, since it is the EPA and State of Montana reference method for measuring

ambient air lead concentrations. One sight (Dudley) was equipped with a dichotomous sampler that measures the fine particles (less than 2.5 microns in diameter) and coarse particles (less than 15 microns but greater than 2.5 microns) in the atmosphere.

FIGURE 2

Ambient Air Monitoring Locations: (1) Dartman, (2) Dudley, (3) Firehall, (4) Hadfield, (5) Hastie, (6) Schneider, (7) South, (8) Townsend (control area in Helena not shown in figure).



2.8 Special Studies--Sampling

2.8.1 Hair, Urine, and Handwash Analyses of Children

One set of special studies conducted during the study included the collection of hair and urine samples from 25 randomly selected Area 1 and 25 randomly selected Area 3 children. These measures were done to evaluate arsenic and cadmium burdens in children living near the lead smelter.

Additionally, children in all study areas participated in a special handwash study to characterize the heavy metal concentrations on their hands during normal play.

2.8.2 Stool Analyses of Children

On a volunteer basis, diapered children were identified for the administration of a questionnaire to their parents and for a 3-day collection of stool materials. These children were a subset of the general sample population. About 68 children participated in this effort to determine soil ingestion by children on the basis of the intake of aluminum, silicon, and titanium, which are predominantly earth crustal elements. The materials, methods, results, and discussion of these stool analyses are in Appendix 23.

2.8.3 Blood and Urine Analyses of Adults

Twenty-five adults each from Areas 1 and 3, identified from census forms as having gardens, having lived within the area for a number of years, and not being industrial workers, were randomly selected for collection of blood and urine samples. This work was done to measure the effects of lead, cadmium, and arsenic accumulations within the adult body.

2.8.4 Indoor Air Analyses

In six randomly selected Area 1 homes, intensive indoor sampling was done to measure inhalable and respirable particulates, to determine the origins of such particles indoor or outdoor, and to state, on the basis of a chemical-mass-balance fingerprinting technique, the percentage each contributed.

2.8.5 3-Inch Soil Samples

For comparison with soil samples collected in the Dallas Lead Study*, 3-inch soil cores were collected at 13% of the front and side yard sampling sites. At these randomly selected sites, 1- and 3-inch cores were collected from adjacent positions and stored separately for analysis.

*Report of the Dallas Area Lead Assessment Study. Lead Smelters Study Group, Office of Toxics Integration. U.S. Environmental Protection Agency, February 1, 1983.

2.9 Laboratory Methods

2.9.1 Biologic Samples

Analytical methods for biologic samples are described in Appendix 20. Ranges of expected results from normal individuals are also described in Appendix 20.

2.9.2 Environmental Samples

Sample preparation and analytical methods used for environmental samples are given in Appendix 18. The procedures followed for the SPEX swing grinder and the SPEX X-Press were identical for soil and vacuum-bag samples. A higher percentage of less than 5-gram samples were, however, recovered from vacuum-bag samples in contrast to soil samples, and the ratio of grinding aid (stearic acid) to sample mass had to be calculated more frequently.

2.10 Quality Assurance

2.10.1 Sampling Procedures--Labels, Logs, Audits, and Custody

All samples were documented with unique labels that CDC prepared before the study (Appendix 19). One sheet of peel-off labels for each household contained labels for all samples, forms, and questionnaires for that household. Photocopies of the original label sheets were used as an accounting system for which samples, forms, and questionnaires had been obtained for each household. These photocopies were placed in a binder before the original label sheets were given to the field teams. The first three digits (312) designated the Montana study; the second three digits, beginning at 001, designated the household. Other parts of the 10-digit CDC numbering system defined the sample parameter, collection method, child number within the family, and internal CDC number audit. In addition, MDHES later added additional alphanumeric symbols to identify analytical methods, instrument types, and sample types (whether a duplicate, blank, or control) for the environmental and biological samples. CDC household numbers did not automatically identify the study area, as did the MDHES census numbering system where the numbers 001 to 199 designated households in Area 1; 200 to 299, households in Area 2; and 300 +, households in Area 3. The two numbering systems were later merged.

As a field team member accomplished a task (obtaining consent, completing a questionnaire, collecting hair, etc.), he or she attached the corresponding peel-off label to the

completed form or sample bag. After a household visit had been completed, absent labels from the sheet gave a quick summary of what samples the team had collected. All team members were instructed to report to the operations center at the end of the day and to remain present until the chain-of-custody clerk had recorded all samples. One member from each team was responsible for submitting the household samples and forms collected during the day. This team member submitted these samples and forms to the chain-of-custody clerk, who logged in receipt of the samples and forms by marking the appropriate labels on the photocopied label sheets. Both the team member submitting the samples and the chain-of-custody clerk signed the photocopied label sheets for each submission. Thus, the photocopied label sheets, kept in a binder, served as a record of chain of custody.

In addition to the above record, a master logbook containing a numerically ordered line listing of households and their sampling status was maintained. This logbook facilitated measuring study progress in terms of total numbers of children sampled.

Within 24 hours of a team's return to the operations center, an auditor (usually an on-site CDC official) reviewed for completeness the forms and questionnaires submitted and accounted for the number of samples collected. After being submitted to the operations center, samples were always locked in cabinets or in holding rooms between analyses. Dryers were securely locked to prevent the samples' being altered in any way during the drying process. Files, logs, and questionnaires were all kept in locked cabinets or drawers. Only supervisory study personnel had access to this information.

2.10.2 Duplicate and Blank Sample Collection

Thirteen percent of the eligible households provided duplicate and blank samples. These homes were selected and the assigned to field sampling teams on a random basis. No single household provided duplicates for all samples because of the difficulties, for example, in obtaining more than one vacutainer of blood from most children. Duplicate soil, floor-wipe, and paint X-ray fluorescence (XRF) readings, however, were easily collected.

2.10.3 Laboratory Analyses of Biologic Samples

ESA Laboratories in Bedford, Massachusetts, performed, on samples from all study children, whole blood lead analyses by anodic stripping voltametry, erythrocyte protoporphyrin analyses by extraction, and hemoglobin analyses. As part of

their normal operating procedure, ESA Laboratories participated in nine external quality control programs for blood lead and EP measurements. During this time, ESA also provided reference laboratory services for five other blood lead testing programs. ESA performed duplicate analyses of blood lead and EP for each sample submitted. Whenever the results of these duplicate analyses differed by more than $\pm 10\%$, the analyses were repeated. For additional quality control, ESA inserted spiked, reference, blank, and pooled samples into its sample stream at the rate of 15%.

During field collection, CDC furnished MDHES with 50 vacutainer tubes of cow (bovine) blood with known lead concentrations. MDHES randomly inserted these samples with those from study participants that were sent to ESA for analysis. Bovine blood lead concentrations were 5-6 times higher than the children's blood lead levels, however, thereby limiting their usefulness for quality control. To provide alternative assessments of ESA quality control, MDHES sent (1) 29 duplicate samples to ASARCO, Inc.'s Salt Lake City laboratory for blood lead analyses, (2) Two blood samples from local horses (equine) to both ESA and ASARCO laboratories, and (3) 25 duplicate samples to CDC for blood lead analyses. The following table gives the results of the analyses at ESA and ASARCO laboratories. No statistically significant difference was found between the children's mean blood lead levels ($N = 28$) reported by ESA and ASARCO ($t = 0.1779$, d.f. = 27, p less than 0.05). The CDC analyses are discussed in section 3.1. Detailed information on CDC quality control for lead and nonlead analytes is in Appendix 20.

Comparison of Blood Lead Levels (ug/dl)
 Analyzed by ESA, Inc., Laboratories, Bedford, Massachusetts,
 and ASARCO, Inc., Salt Lake City, Utah

ESA	ASARCO	ESA	ASARCO
7	6	8	8
12	9	2	8
6*	7*	5	8
10	10	24	24
19	18	6	8
9	8	6	8
9	6	11	10
58**	57**	10	10
8	8	10	10
10	10	9	10
6	8	6*	4*
11	9	9	7
8	9	7	8
7	5	8	11
7	10	12	9
10	9		

* Equine Sample

** Bovine Sample

CDC measured lead and erythrocyte protoporphyrin in whole blood in duplicate on 7% of the blood samples collected from children participating in the study.

In addition to this quality control service, CDC conducted the primary analyses in this study for cadmium in blood; arsenic, lead, cadmium, beta-2-microglobulin, creatinine, and protein in urine; and arsenic, cadmium, and lead in hair. Analytical methods and quality control assurance for these duplicate and primary analyses are described in Appendix 20.

2.10.4 Laboratory Analyses of Environmental Samples

MDHES repeated its chemical analyses at a 10% level for vegetable, floor-wipe and handwash samples, and at a 5% level for soil and vacuum-bag dust samples.

Control limits for measuring lead in soil were based on the recovery of 90% to 110% of the National Bureau of Standards (NBS) reference materials 1645 (River Sediment) and 1648 (Urban Particulates). Similar limits ($\pm 10\%$ of the target concentration) were also used for nonsoil environmental samples.

The following table summarizes the blind quality assurance for environmental lead analyses. Precision is expressed as the coefficient of variation, except for the air lead data set. Accuracy is measured in percent recovery of reference material.

Environmental Lead Quality Assurance for Blind Samples

Parameter	Method*	Number Samples	Precision (CV)	Accuracy (% Recovery)
Handwash & Floor wipe	ICP	16	11.4	93.8
	ICP	16	11.4	93.8
Vegetation	ICP	5	16.6	96.4
Soils & Vacuum Dust	XRF	31	0.85	95.2
Air Lead	ICP	22	(-1.59%)	101.3

*ICP = Inductively coupled argon plasma emission spectroscopy
XRF = X-ray fluorescence

The handwash and floor-wipe samples listed in the above table were analyzed as blind environmental samples by using EPA-certified reference solutions. Fewer vegetation samples were run, since 25 similar samples were sent to an EPA contract laboratory for quality control (QC) purposes. Low recoveries for blind NBS samples by the EPA contract laboratory discredited its analyses of these vegetation samples; therefore, the results of those analyses are not reported here. MDHES analyzed NBS orchard leaf reference materials to obtain the above vegetation data. Soils and residential vacuum dust were compared with NBS river sediment material. Air lead precision and accuracy data came from collocated monitors and from instrument calibrations, respectively. Minimum detection limits were: 0.05 mg/l for handwash samples; 0.05 mg/l for floor-wipe samples; 2.0 ppm for vegetation samples; 5.1 ppm for soil and vacuum dust samples; and 0.05 $\mu\text{g}/\text{m}^3$ for air lead samples.

Additional quality assurance data and the forms used by MDHES for internal laboratory chain of custody are in Appendix 21.

EPA repeated laboratory analyses on 11% of the soil, vegetation, and stool samples analyzed by MDHES. For quality assurance of ambient air sampling, EPA conducted a systems and performance audit during September 12-15, 1983, of the particulate sampling network used in this study. The method and results of this audit are included in this report as Appendix 22.

2.11 Data Handling

Questionnaires, environmental sample collection forms, and laboratory result forms were precoded for computer data entry. To the 10-digit CDC identification number, MDHES added four columns for sample and method identifiers and nine additional columns for the data itself.

Blood lead, hemoglobin, and erythrocyte protoporphyrin results received from ESA, Inc., were keypunched for transfer to IBM disc file storage. No personal identifying information, such as name or street address, was keypunched. MDHES performed extensive data verifications on these keypunched values to insure the accuracy of all data before transferring them to IBM disc file storage.

MDHES electronically transferred unedited ICP (inductively coupled argon plasma) laboratory data to magnetic tapes from ICP magnetic diskettes. Transferred data was then edited to remove standards, internal quality control information, duplicate samples, and blanks from the data file. Laboratory X-ray fluorescence data were keypunched and stored on magnetic tape. Data from the hand-held XRF analyzers were

keypunched from the reporting forms the field team used. MDHES shipped edited data tapes containing blood lead levels and environmental data to CDC in Atlanta.

CDC keypunched questionnaire data and transferred these data to magnetic tape. Data was edited and merged with the blood lead and environmental data received from MDHES. The final tape contained a separate file for each child who participated in the study; it listed information from his or her questionnaire, biologic samples, and environmental samples.

MDHES conducted data analyses using the Statistical Package for Social Sciences (SPSS). CDC conducted data analyses using the Statistical Analysis System.

3.0 Results

3.1 Blood Lead and Erythrocyte Protoporphyrin Levels

The numbers of eligible children, i.e., those who met the age and residence criteria, were 104 in Area 1, 254 in Area 2, and 79 in Area 3--a total of 437 eligible children. Participation rates were 97% in Area 1, 94% in Area 2, and 76% in Area 3,--an overall participation rate of 91%.

Ninety-eight Area 1, 237 Area 2, and 61 Area 3 children had their blood lead (BL) levels determined. The BL levels ranged from 1 to 33 micrograms per deciliter (ug/dl). Analyses of blood lead levels were within acceptable quality control limits at ESA Laboratories during the months in which these samples were analyzed, as documented by ESA performance in the CDC blood lead proficiency testing program (Table 1). Blood samples from 25 children were analyzed by both ESA Laboratories and CDC. As expected, the natural log transformations of the blood lead levels reported by ESA Laboratories were significantly less than those CDC reported (paired-comparisons t test: $t = -6.56$, d.f. = 24, $p = 0.0001$). The 3.56 ug/dl difference between the geometric mean blood lead ESA Laboratories reported (7.77 ug/dl) and that CDC reported (11.33 ug/dl) was about that typically found between anodic stripping voltametry (ASV), which ESA Laboratories use, and graphite furnace atomic absorption spectroscopy (AAS), which CDC uses⁸. This difference was in the direction expected, with the ASV method consistently giving lower values than the AAS method. When CDC blood lead results were regressed on ESA results, a plot of the predicted versus the residual values showed the laboratories to be in better agreement as blood lead levels increased.

The geometric mean BL level was 12 ug/dl for Area 1 children, 9 for Area 2 children, and 6 for Area 3 children (Table 2). Natural log-transformations of individual BL values were used in all statistical comparisons among study areas to normalize BL and EP distributions. Mean log-transformed BL values were statistically significantly different among the three study areas, as determined by analysis of variance ($F = 41.08$, $p = 0.0001$).

Ninety-eight Area 1, 235 Area 2, and 61 Area 3 children had their erythrocyte protoporphyrin (EP) levels determined. The EP levels ranged from 8 to 77 ug/dl. Analyses of EP levels were within acceptable quality control limits at ESA Laboratories during the months in which these samples were analyzed, as documented by ESA performance in the CDC EP proficiency testing program (Table 3). Blood samples from 25 children were analyzed by both ESA Laboratories and CDC. Natural log-transformations of the EP levels reported by CDC were significantly less than those reported by ESA Laboratories (paired-comparisons t test: $t = -7.05$, $d.f. = 24$, $p = 0.0001$). This discrepancy is likely to be due to the EP level's being relatively close to the detection limits of both laboratories. For the 25 shared samples, the reported geometric mean EP levels were 16.7 ug/dl (CDC) and 19.6 ug/dl (ESA).

The geometric mean EP levels were 21, 20, and 19 ug/dl for Area 1, 2, and 3 children, respectively (Table 4). Mean log-transformed EP values were statistically similar among the three study areas as determined by analysis of variance ($F = 0.97$, $p = 0.38$).

An examination of the BL and EP results by age shows that higher mean values generally occurred in younger children in Area 1 (Table 5).

Four major risk classifications have been established for children who have been exposed to lead². These are based on EP and BL levels and have been useful in setting priorities for medical evaluation of screening results. No children were identified to have either of the two highest risk classifications (Class III or IV). One child, from Area 1, was classified as having lead toxicity, i.e., this child had a BL greater than 25 ug/dl and an EP greater than 35 ug/dl (Table 6). The actual BL and EP values of this child were 33 ug/dl and 53 ug/dl, respectively.

3.2 Soil Levels of Lead and Other Metals

Table 7 gives the numbers of household premises from which various soil samples were collected. The number of households with eligible children that participated in the

study was 73 in Area 1, 179 in Area 2, and 44 in Area 3,--a total of 296. Table 7 shows that the soil lead levels ranged from 3 to 7,964 ppm in Area 1; 3 to 6,030 ppm in Area 2; and 28 to 500 ppm in Area 3. These results indicate that the distribution of soils with high lead levels is not uniform throughout the valley. Analyses of variance for different soil sample types show that the mean log-transformed soil lead levels are statistically significantly different among the three study areas for front yard and back yard composite samples ($F = 110.0$, $p = 0.0001$); for side yard samples ($F = 75.4$, $p = 0.0001$); for play area samples ($F = 14.6$, $p = 0.0001$); and for garden samples ($F = 32.5$, $p = 0.0001$).

Table 8 displays the relationship between the levels of lead and other metals found in the 1-inch and 3-inch core samples collected from adjacent positions within individual yards. The coefficients of these comparisons show a high degree of correlation between 1- and 3-inch soil core samples collected from front yards in terms of the levels of all metals tested. The correlations among side yard samples were less, but still statistically significant.

Table 9 displays the results of statistical tests of the differences in the concentrations of lead and other metals found in 1- and 3-inch soil cores. No statistically significant difference was found between the lead levels in 1- and 3-inch soil cores collected in front yards and side yards. Table 10 displays the ranges of concentrations of lead and nonlead metals that would be expected in uncontaminated soils.⁹

3.3 Dust Lead Levels

Grab samples of household dust were collected from 179 vacuum cleaners (Table 11). The lead levels in these samples ranged from 80 to 18,361 ppm and were highest in samples from Area 1 and lowest in samples from Area 3. Analysis of variance shows that the mean log-transformed lead levels of these samples are statistically significantly different among the three study areas ($F = 57.0$, $p = 0.0001$). The geometric mean lead levels in vacuum-bag dust were slightly more than twice the corresponding levels in front yard and back yard soils in Areas 1 and 2. In Area 3 vacuum-bag dust lead levels were more than four times higher than the levels in front yard and back yard soils.

Floor-wipe samples showed low levels of lead in all three study areas (Table 11). These levels approach the limits of detection of the instrumentation used (Table 12).

Despite the greater variation that measurements near instrumental detection limits tend to exhibit, analysis of

variance of the log-transformed mean lead levels in floor-wipe samples showed statistically significant differences among the three study areas ($F = 34.7$, $p = 0.0001$). Lead levels found in the household dust samples collected from one square meter of carpet are summarized in Table 13.

3.4 Lead Levels in Garden Vegetables

Up to three garden vegetable samples were obtained by the sampling teams from each garden sampled. The samples normally consisted of leafy vegetables such as lettuce, when available, or root vegetables such as radishes and carrots. No distinction, however, was made between leafy and root vegetables during collection or during analysis. Thus the lead levels of all vegetables sampled within any study area were combined for statistical analyses (Table 14).

One vegetable sample in Area 1 had a lead value of 6,380 ppm. This outlier was excluded in calculating the statistics listed in Table 14.

3.5 Lead-Based Paint

Painted surfaces at 296 residences were tested for the presence of lead-based paint. XRF calibration readings were out of range for 194 of these residences (Table 15); therefore, the surface readings from these homes were excluded from the following summary statistics. Of the 1,381 surfaces tested with accurately calibrated XRF analyzers, 1,260 (91%) did not contain detectable levels of lead-based paint (i.e., levels were less than 0.7 mg lead per square centimeter). Specifically, the percentage of surfaces with negative lead readings was 84% in Area 1, 96% in Area 2, and 89% in Area 3. Over all three areas, 77 surfaces had a low amount of lead, and 44 had a moderate to high amount. These results are summarized in Table 16.

3.6 Ambient Air Analysis

Ambient air lead readings were adjusted to 25°C and 760 mm of mercury for purposes of standardizing temperature and atmospheric pressure.

Table 17 presents a summary of the ambient air quality data collected during the study; the summary includes the mean levels for July, August, and September, and the mean for the three months combined.

Readings at all four monitoring stations in Area 1 and at one of the three stations in Area 2 (Dartman) exceeded the ambient air quality standard for lead (1.5 ug per cubic meter). The other sites were well within this standard over the 3-month period.

The Area 1 concentrations of lead were substantially higher than concentrations for all Area 2 monitors. Similarly, Area 2 lead readings exceeded those of Area 3. Interestingly, the Dudley sampling site values (Area 2) were nearly as low as those of the Area 3 site. Within Area 1, the Firehall sampling site ambient lead readings were exceptionally high, being nearly three times greater than the ambient air quality standard for lead.

3.7 Water Lead Levels

MDHES Water Quality Bureau measurements showed that East Helena drinking water samples had lead concentrations less than 0.005 ug/l in July 1983. This finding was consistent with measurements of the same water supply in July 1982. Drinking water wells north of the wells that supply East Helena drinking water also had lead concentrations less than 0.005 ug/l in July 1982. Several wells east of East Helena had trace lead concentrations (i.e., less than or equal to 0.010 ug/l) in July 1982. Helena drinking water supplied from the Missouri River had lead concentrations less than 0.005 ug/l in both July 1980 and July 1984. Thus, Areas 1 and 2 (East Helena and its surroundings) and Area 3 (Helena) have drinking water supplies with similarly low levels of lead contamination.

3.8 Handwash Analyses

The original protocol called for a sample from each child in Area 1 and every other child in Area 3. This was modified during the actual sampling to include every child in all three areas because of the relative ease with which both sampling and laboratory analyses could be done. Table 18 provides a summary of the handwash data by element and area. Fifty percent of the handwash concentrations were at or below instrumental detection limits for lead (Table 19).

3.9 Comparisons of Original and Duplicate Environmental Samples

Tables 20 and 21 display comparison statistics for original and duplicate floor-wipe samples and front yard soil samples. As shown in these tables, high correlations were found between the levels of lead in the original and duplicate samples taken from individual households, and no statistically significant differences were found between the mean lead levels of original and duplicate samples.

3.10 Correlations Among Environmental Variables

Table 22 shows Pearson correlation coefficients for the lead levels found in four types of soil samples and one type of dust sample collected in all study areas. Significant

correlations exist between: (1) the log-transformed lead levels found in front yards and back yard composite soil samples and those found in side yard soil samples, play area soil samples, vacuum-bag dust grab samples, and garden soil samples; (2) the log-transformed lead levels found in side yard soil samples and those found in play area soil samples, vacuum-bag dust grab samples, and garden soil samples; (3) the log-transformed lead levels found in play area soils and those found in vacuum-bag dust grab samples and garden soil samples; and (4) the log-transformed lead levels found in vacuum-bag dust grab samples and those found in garden soil samples.

3.11 Associations of Environmental Lead Levels and Questionnaire Variables With Blood Lead Levels

3.11.1 Soil Lead and Yard-Grass Coverage

Table 23 displays the mean blood lead levels of children within each of the three study areas according to the lead levels found in composite samples of front yard and back yard soils. Within Area 1, for soil lead categories having more than three children, children with the most highly contaminated yard soils (1,501-3,500 ppm) had the highest mean blood lead level. In addition, within Area 1, children with lesser contaminated front yard and back yard soils tended generally to exhibit a dose response effect between their soil-lead categories and mean blood lead levels.

Table 24 displays the mean EP levels within each study area according to the lead levels found in the composited samples of front and back yard soils. No trends of association between EP level and soil lead level are apparent from these data.

Table 25 displays the mean ages in years of the children whose blood lead levels were categorized in Table 17. In Areas 1 and 2, mean ages were similar across all categories having more than five children. Mean ages for these groups ranged between 3.2 and 4.1 years.

Table 26 provides the numbers of children within each study area who had front yards and back yards with less than 50% grass coverage and with 50% or more grass coverage. Comparison of the proportions within both categories shows no statistically significant difference across the three study areas (Chi-square = 1.64, $p = 0.44$).

Table 27 provides the mean blood lead levels of children within each study area according to four categories of the percentage of the front yards and back yards covered by grass. In each area, few children had yards with less than

75% grass coverage. This skewed distribution limits evaluation of how the mean blood lead level might vary with respect to yard grass coverage.

Table 28 displays the mean blood lead levels of children within each area according to the lead content found in soil samples from their side yards. Within Area 1, children with the most highly contaminated side yard soils (1,501-8,000 ppm) had the highest mean blood lead level. Within both Areas 1 and 2, children with lesser contaminated side yard soils tended to exhibit dose-response effects between their soil lead categories and mean blood lead levels.

3.11.2 House-Dust Lead

Table 29 displays the mean blood lead levels of children within each area according to the lead content of vacuum-bag dust grab samples. Within both Areas 1 and 2 children with higher vacuum-bag dust lead levels tended, in general, to have higher mean blood lead levels. Table 30 provides the numbers of households sampled within each area according to these categories of vacuum-bag dust lead.

Table 31 provides the mean blood lead levels of children within each study area according to the levels of lead found in floor dust wipe samples. As presented earlier in Table 12, about 40% of all floor wipe samples collected had lead concentrations below detection limits. Nevertheless, for those samples with detectable levels, higher levels are associated with higher mean blood lead levels in both Areas 1 and 2 (Table 31).

3.11.3 Lead-Related Hobbies

Table 32 provides the number of children within each study area who lived in households where at least one lead-related hobby was practiced and the number who lived in households where no lead-related hobby was practiced. Comparisons of the proportions of children in these categories shows no statistically significant difference across the three study areas ($\chi^2 = 1.537$, $p = 0.46$).

Table 33 displays the mean blood lead levels of children within each area according to the presence and absence of lead-related hobbies in the children's homes. Table 34 displays the mean EP levels of children within each study area according to the presence and absence of lead-related hobbies in their homes. As shown by these two tables, children who lived in households where no lead-related hobby was practiced tended to have lower blood lead levels, although this difference was only statistically significant in Area 3, where both groups of children had similar EP levels.

3.11.4 Storm Windows

Table 35 provides the number of children within each study area who lived in houses that had storm windows and the number who lived in houses that did not have storm windows. Comparison of the proportions in these two categories shows no statistically significant difference across the three study areas ($\text{Chi-square} = 0.79$, $p = 0.67$).

Table 36 displays the mean blood lead levels of children within each study area according to the household's use of storm windows. No statistically significant difference was found between the mean blood lead levels of children living in households with and without storm windows in any study area. Table 36 lists the relevant statistics for these within-area comparisons of mean blood lead levels. Table 37 displays the mean EP levels within each study area according to household storm window use. As with blood lead levels, no statistically significant difference was found between the mean EP levels of children living in households with and without storm windows in any study area. Table 37 lists the relevant statistics for these within-area comparisons of mean EP levels.

Table 38 provides the mean house-dust lead levels according to household storm window use within each area. No statistically significant difference was found between the dust lead levels in houses with and without storm windows. Table 38 displays the relevant statistics for these within-area comparisons of dust lead levels.

3.11.5 Neighborhood-Grown Produce

Table 39 shows the number of children within each study area who frequently ate neighborhood-grown fruits or vegetables and the number of children who did not frequently eat such neighborhood produce. Comparison of the proportions of children in these two categories shows no statistically significant difference across the three study areas ($\text{Chi-square} = 1.816$, $p = 0.40$). Similarly, no statistically significant difference was found between the mean blood lead levels of children within any area according to the children's frequencies of eating neighborhood-grown fruits or vegetables (Table 40). In Area 2, the mean EP level of children who frequently ate neighborhood produce was significantly lower than the mean EP level of children who did not frequently eat neighborhood produce ($p = 0.001$, Table 41).

3.11.6 Dietary Supplements

Table 42 shows the number of children in each study area who were taking some sort of dietary supplements and the number of children who were not. Comparison of the proportions of children within these two categories shows that proportionately more children tended to take dietary supplements in Area 3. This difference in proportions, however, was not statistically significant (Chi-square = 5.70, $p = 0.058$). Comparisons of the mean blood lead levels and mean EP levels of the children who did and did not take dietary supplements showed no statistically significant differences within any study area (Tables 43 and 44).

3.11.7 Play Surface Type

Table 45 provides the number of children in each study area who played on grassy surfaces and the number who played on nongrassy surfaces such as concrete, asphalt, dirt, or sand. Comparison of the proportions of children in these two categories shows no statistically significant difference across the three study areas (Chi-square = 0.827, $p = 0.66$). In Area 1, the mean blood lead and the mean EP level of children who played on nongrassy surfaces were significantly lower than the corresponding levels of children who played on grassy surfaces (Tables 46 and 47).

3.11.8 Household Member Smoking

Table 48 shows the number of children in each area who lived in households where someone smoked and the number who lived in households where nobody smoked. Comparison of the numbers of children in these two categories across all three study areas shows that proportionately fewer children in Area 3 tended to live in households where someone smoked, although this difference was not statistically significant (Chi-square = 5.63, $p = 0.06$). In both Areas 1 and 2, the mean blood lead levels of children who lived in households where nobody smoked were significantly lower than the levels of children who lived in households where someone did smoke (Table 49). Conversely, in Area 3, the mean EP level of children who lived in households where nobody smoked was higher than the corresponding level of children who lived in households where someone smoked (Table 50).

3.11.9 Habits of Taking Food Outside

Table 51 shows the number of children in each area who often took food outside and the number who did not often take food outside. Comparison of the proportions of children in these

two groups across all three study areas shows no statistically significant difference (Chi-square = 1.65, $p = 0.44$). No statistically significant difference was found between either the mean blood lead levels or the mean EP levels of children in these two groups in any study area (Tables 52 and 53).

3.11.10 Mouthing Habits

Table 54 displays the number of children in each area who used a pacifier, sucked their thumbs, or chewed fingernails, as well as the number of children who did not have these habits. Comparison of the proportions of children in these two groups across all three study areas shows no statistically significant difference (Chi-square = 1.49, $p = 0.48$). Comparisons of the log-transformed mean blood lead levels and log-transformed mean EP levels of the children in these two categories showed no statistically significant differences within any study area (Tables 55 and 56).

3.11.11 Lead Paint

Tables 57 and 58 classify the mean blood lead levels of children within each study area according to (1) whether lead was detected by X-ray fluorescence (XRF) on any interior or exterior surface of the child's home and (2) whether lead paint was detected along with a chipping or peeling surface in the home. Within Areas 1 and 2, mean blood lead levels were similar for (1) children living in homes with and without detectable lead paint and (2) children living in homes with and without detectable lead paint that was chipping or peeling. In Area 3, however, children in homes with detectable lead paint had blood lead levels that were significantly higher than those of children in homes with no detectable lead paint. In Area 3, all homes found to have lead paint also had chipping or peeling paint.

3.11.12 Sibling Analyses

Similarly aged siblings exposed to a similar environment can be expected to have similar blood lead levels. To investigate this collinearity, we tested the blood lead levels of all sibling pairs having age differences of 3 years or less by correlation analyses. Because the study population contained children aged 1 through 5 years, sibling pairs could range from 1- to 4-year-old comparisons up to 2- to 5-year-old comparisons. Table 59 shows the correlations of blood lead levels in sibling pairs whose ages differed by 1 year or less, 2 years or less, and 3 years or less. As the table shows, the correlation of sibling blood lead levels is substantial for all three types of sibling pairs.

3.11.13 Model for Predicting Children's Blood Lead Levels

Our major goal in constructing this model was to determine if the lead levels in soil and house dust were significantly related to children's blood lead levels after we had accounted for the effects of other variables known or suspected to cause elevations in blood lead. Well-documented correlates of blood lead levels in children include environmental sources, such as leaded paint, lead derived from home hobbies, lead in food, and lead in ambient air. Likely correlates of blood lead levels in children include the children's play behavior, their locations of play, the intensity of their play, their mouthing behaviors, the characteristics of their houses, their nutrition, and certain general characteristics of their families.

Sample Size Considerations

Given the large number of questionnaire and environmental data items that the study attempted to collect for each child, various items are missing from the records for several children. Thus, regression analyses, which consider all variables jointly, exhibit substantial reduction in workable sample size because of the scattered nature of missing data. Therefore, when univariate analysis showed a large proportion of missing data and when no association between the variable and blood lead levels was apparent, the variable was excluded from regression analyses.

Further, the study population of children with measurable blood lead levels necessarily restricted the number of variables to be considered in a regression analysis. To reduce the number of independent variables, two composite variables were formed by using principal components analyses.

Principal Components

Six questions from the study questionnaire about children's mouthing behaviors and one about their habits of eating food outdoors were selected for analysis on the basis of having sufficient numbers of responses. For any given child, the responses to the questions within this set of variables are likely to be significantly correlated. To reduce the dimensionality of the system of independent variables for regression analysis and to create uncorrelated vector variables for this set of presumably correlated variables, we used principal component analysis to transform the set of mouthing behavior variables.

An important feature of principal components is that although the complete set of principal components will reproduce the correlation matrix exactly and will thus account for all the variance in the vector variable, a subset of the principal components can be retained. This subset will extract more of the variance of the vector variable than any other set of n orthogonal factors.

Table 60 shows the first three principal components or factors formed by using the eight mouthing questions. The weightings in Table 60 indicate that factor MOUTH 1 scores highest for children who put things other than food in their mouths and who put their mouths on furniture or window sills. Factor MOUTH 2 loads heaviest on mouthing furniture and paint chips.

Together, the first two components or factors account for 59% of the variance in the seven-question battery. Both of these component vector variables were entered into the regression analyses as independent variables. This reduced the dimensionality of the regressions and retained most of the information contained in the entire battery of mouthing questions.

Statistical Procedures

Multiple linear regression models were used to determine which independent variables were significantly related to the natural logarithm of blood lead. The general procedure for independent variable selection was as follows. Variables representing each possible pathway of exposure were analyzed jointly with no interaction effects or second order terms included. Principal component vectors were then included in the regression equations. Variables that were significantly related to blood lead were retained for more intensive analysis. After first-order effects were examined and the dimensionality of the regression functions was reduced, selected higher order terms and interaction effects were introduced. Age was modelled as a single variable and as age and age-squared. No analyses were conducted with only one member from each age-similar sibling pair because of the insufficient sample size that would result.

Computer programs available through the Statistical Analysis System (SAS) were used for these analyses. PROC STEPWISE was used for variable selection by backward elimination and also by maximum R^2 improvement (MAXR). PROC REG was used to confirm the joint relationship among variables.

A final set of multiple regression analyses aimed specifically at estimating the independent contribution to blood lead from lead in soil or in dust, or in soil and dust together, was performed (Table 61). The variables used in these models are defined in Table 62.

To test for the influence of hand-to-mouth activity, we performed two more regression analyses. Each consisted of the independent variables found to contribute the most to blood lead in the above series and an age interaction term. The first of these last two models involved an interaction term between age and soil lead. The second involved an interaction term between age and dust lead. Table 63 gives a complete description of both models and how their respective interaction terms were constructed.

Regression Results

As Table 61 shows, the regression coefficient for soil lead diminishes from Model 1 to Model 4 to Model 6, as air lead, questionnaire data, and dust lead are incorporated in the models. In Model 7, when the variables designating home location are added to the model, soil lead is no longer a statistically significant contributor to the variance in children's blood lead levels. Conversely, the regression coefficient and the statistical significance of dust lead remain relatively constant from Model 2 to Model 5 to Model 6 to Model 7, as air lead, questionnaire data, soil lead, and home location are added to the model. Finally, Area 1, the variable designating that the child's home was located within 1 mile of the smelter, appears as a significant contributor to blood lead level in both the backward stepwise elimination and the maximum R^2 stepwise improvement regressions for Model 7.

As Table 63 shows, neither the interaction term of age and soil lead nor the interaction term of age and dust lead appeared as a significant contributor to blood lead.

The final multiple regression model for this study incorporates dust lead levels but not soil lead levels (Table 64). The model also incorporates the ambient air lead level and the variables that designate location of the child's home in Area 1 and the presence of a smoker in the child's home. None of the other questionnaire variables appears significantly in the model as a main effect, as an interaction, or as a composite factor.

3.12 Levels of Other Metals in Blood, Urine, and Hair and Their Associations with Questionnaire Variables

3.12.1 Children

Laboratory and questionnaire data were available for 36 children living in Areas 1 and 3. Table 65 displays the mean blood level of cadmium, the mean urine levels of lead and arsenic, and the mean hair levels of lead, cadmium, and arsenic found for children in both areas. Normal ranges for these analytes are shown in Appendix 20. As Table 65 shows, the mean hair lead level was significantly higher for children living in Area 1.

Results of analyses of these levels of metals according to selected questionnaire variables are summarized in Tables 66-68. Table 66 shows the mean levels of these metals in blood, urine, and hair according to the absence and presence of storm windows in the childrens' homes within Areas 1 and 3. No statistically significant differences were found within either study area between the levels for children living in houses without and with storm windows. Table 67 displays the mean levels of metals in blood, urine, and hair according to the type of surface on which the children played outdoors. No statistically significant differences were found within either study area between the levels for children who played mainly on grassy surfaces and those who played mainly on nongrassy surfaces, such as dirt, asphalt, and concrete. Table 68 displays the mean levels in blood, urine, and hair according to the absence and presence of a household member who smoked. In Area 3, children who lived in households where someone smoked had a significantly higher mean hair arsenic level than children who lived in households where nobody smoked.

3.12.2 Adults

Laboratory and questionnaire data were available for 33 adults living in separate residences in Areas 1 and 3. Of 17 adult participants in Area 1, 1 was male and 16 were female. Similarly, of 16 adult participants in Area 3, 1 was male and 15 were female. Table 69 displays the mean blood level of cadmium and the mean urine levels of cadmium and arsenic found for adults in both areas. Normal ranges for these analytes are given in Appendix 20. No significant difference was found between the adults in Areas 1 and 3 with respect to mean blood cadmium levels, mean urine cadmium levels, or mean urine arsenic levels.

Table 70 displays the mean ages and mean lengths of continuous residence in the homes where the interviews were conducted for adults in both study areas. No significant difference in mean age or length of continuous residence was found between the adults in Areas 1 and 3.

Results of analyses of the levels of metals in adult blood and urine according to selected questionnaire variables are summarized in Tables 71-75. In Area 1, no significant differences were found between the mean levels of (a) adults who had worked in a lead-related industry or whose spouses had worked in a lead-related industry and (b) adults who had not worked in a lead-related industry and whose spouses similarly had not had such occupations (Table 71). In Area 3, only one adult participant had a history of having worked in a lead-related industry. Thus, no statistical comparisons of mean levels of metals in adult blood and urine samples were done in Area 3 according to lead job history. In Area 1, only one adult participant reported no gardening or yardworking activities. Thus, no statistical comparisons of mean levels of metals in adult blood and urine samples were done in Area 1 according to yardworking habits. In Area 3, where such statistical comparisons of mean levels were possible, no significant differences were found between adults who gardened or did yard work and adults who did not (Table 72). In Area 1, only one adult participant reported not eating neighborhood-grown fruits or vegetables. Thus, no statistical comparisons of mean levels of metals in adult blood and urine samples were done in Area 1 according to habits of eating neighborhood produce. In Area 3, where such statistical comparisons of mean levels were possible, no significant differences were found between adults who ate neighborhood produce and adults who did not (Table 73). No significant differences were found in either Areas 1 or 3 between the mean levels of metals in blood and urine samples of adults who ate fish caught locally and adults who did not eat such fish (Table 74). In Area 1, adults who smoked cigarettes and adults who did not had similar mean levels of metals in their blood and urine samples (Table 75). In Area 3, however, adults who smoked cigarettes had a significantly higher mean urine cadmium level than that found for nonsmokers (Table 75).

4.0 Discussion

4.1 Associations Between Environmental Characteristics and Blood Lead Levels

Children living closer to the smelter had higher blood lead levels than children living farther away. The following discussion addresses possible sources of lead to which these children may have been exposed and the associations between these lead sources and the children's blood lead levels.

Soil lead contamination is associated with children's blood lead levels in the Helena Valley, as evidenced by the following observations: (1) highly significant differences exist among the three study areas in the lead levels of all

four types of soil tested, and these differences are mirrored by highly significant area differences in children's blood lead levels; (2) children who have higher concentrations of lead in the soil around their homes are of ages similar to those of children with lower concentrations of lead in the soil around their homes; thus, the positive association between mean blood lead levels and soil lead level categories is not likely to be due to a confounding effect of age and age-related behavioral characteristics.

Higher blood lead levels were found in higher house dust lead categories in Areas 1 and 2, where dust lead levels were determined by both the vacuum-bag dust grab and floor wipe sampling methods. This positive association between house dust lead contamination and children's blood lead levels was likely to have been a result of ambient air lead contamination from current smelter emissions and soil lead contamination from past smelter emissions. This conclusion follows since lead contamination in the dust collected from households in this study could have reflected: (1) ambient air lead from smelting operations or from automobile exhaust; (2) soil lead that enters the house from outside; (3) paint lead that chips or peels off walls or moldings; and (4) lead filings or scraps from lead-related hobbies. Air lead concentrations were markedly different among the three study areas. Because automobile traffic densities were similar in all three areas, the difference in air lead concentrations among these areas is not likely to be due to a difference in automobile exhaust from vehicles using leaded gasoline. Although unacceptable XRF calibration readings limited the number of households for which accurate lead paint data were available, it is not likely that the homes for which no XRF data were available differed from those for which we do have XRF data. The accurately calibrated XRF readings showed that little leaded paint was present and that lead paint on intact or chipping and peeling surfaces had no statistically meaningful association with children's blood lead levels except in the comparison area. With respect to lead-related hobbies, we found that children who lived in households where no lead-related hobby was practiced had significantly lower blood lead levels only in Area 3, the comparison area. Thus, lead from painted surfaces and from hobby activities provided no explanation of blood lead differences within or between Areas 1 and 2, where air and soil lead levels were higher than in Area 3.

These findings also suggest that higher air and soil, and, consequently, dust lead levels obscured whatever contributions lead-based paint and lead-related hobbies may have made to children's lead exposures. Where soil, dust, and air lead levels are low, as in Area 3, exposures to lead-based paint and lead-related hobbies may be more important.

The purpose of the question on storm window use was to evaluate whether children living in houses with storm windows had lower blood lead levels than those living in houses with no storm windows. The assumptions were that: (1) storm windows might decrease the amount of lead-contaminated dust entering the house from outside and thereby might lessen the house-dust lead exposure of children in these homes; and (2) storm windows might provide a surrogate measure of housing quality. Since this study was conducted during August and the first weeks of September, the study participants were not likely to have used storm windows for the 3-month period before the blood sampling. Thus, the only assumption about a possible relationship between storm windows and children's blood lead levels that we were able to evaluate was that of storm windows' being a possible surrogate measure of housing quality. In all three study areas, children living in households with and without storm windows had similar blood lead levels, EP levels, and dust lead levels. Since previous studies of children's lead exposure have shown housing quality to be associated negatively with blood lead levels, the findings suggest two possible explanations: (1) storm windows did not serve as a surrogate measure of housing quality in this study, and (2) the quality of housing was relatively uniform within and between study areas.

Since the home environment and the immediate exterior environment are the same for siblings living in the same household, the results of the correlational analyses of siblings' blood lead levels lend support to the internal validity of the blood lead data. Differences in sibling blood lead levels are likely to reflect greater nutritional and behavioral differences than environmental differences.

4.2 Associations Between Behavioral Characteristics and Blood Lead Levels

Certain behavioral characteristics may predispose children to ingest more lead from various sources in their environments or to absorb more of the lead they do ingest. The following discussion describes the associations found between these behavioral characteristics and children's blood lead levels.

Fruits and vegetables grown in lead-contaminated soils are likely to have lead-contaminated soil particles on their surfaces which may not be entirely removed by routine washing. Thus, children who eat these fruits and vegetables may ingest more lead than those who do not. In this study, however, children's habits of eating fruits and vegetables grown in their neighborhoods had no statistically meaningful association with their blood lead levels in any study area. In addition, an interesting finding was that fewer than 55% of the children within any study area frequently ate locally grown produce.

Animal studies have shown that intestinal lead absorption may: (1) decrease when calcium and certain other minerals are present in the intestine and (2) increase in iron deficiencies. To explore the relationship between children's nutritional status and blood lead levels, we included in the questionnaire for this study questions on the use of vitamins, minerals, and other dietary supplements. The responses to these questions showed that the use of dietary supplements was not statistically different among the three study areas and that children who were taking dietary supplements had blood lead levels similar to those who were not.

Children who usually play on nongrassy surfaces may be likely to have greater exposures to lead-contaminated dust by inhalation and ingestion than children who play on grassy surfaces. In this study, however, children in Area 1 who played on nongrassy surfaces had blood lead levels significantly lower than those of children who played on grassy areas. The inconsistency probably reflects differences other than the surface of the play area.

Children who exhibit more mouthing activities may have greater exposures to lead-contaminated soil or dust particles. These increased exposures result from chewing or sucking on objects that have soil or dust particles on their surfaces. To explore the relationship between children's mouthing activities and their blood lead levels, we included several questions about the children's oral habits. Analyses showed that children in all three study areas had similar habits of taking food outside and that within any study area the levels of those children who did take food outside were similar to the blood lead levels of children who did not. Children who had the habit of often using a pacifier, often sucking their thumbs or fingers, or sometimes chewing on their fingernails and children who did not have these habits had similar blood lead levels in all three study areas. Thus, in summary, we found no associations between these questionnaire variables on mouthing activities and blood lead levels when we looked at the variables individually.

4.3 Model for Predicting Children's Blood Lead Levels

The series of regression analyses (Tables 61 and 63) and the final regression model (Table 64) suggest the following conclusions.

First, the contribution of dust lead to children's blood lead levels is greater, in this sample, than the contribution of soil lead. While the ability to explain variation in the observed blood lead levels is limited to less than 30%, a significant and positive association exists between dust lead levels and blood lead levels. This relationship remains even when other factors known to be related to blood lead are taken into account.

Second, average ambient air lead levels measured within each study area are significantly associated with blood lead levels and have an effect upon blood lead levels in addition to the effect from dust lead. From the results of this study, dust and air lead levels appear to make independent contributions to blood lead levels.

Third, in this sample, the relative contribution from environmental lead sources to blood lead levels greatly outweighs the contribution from play behaviors, age-specific hand-to-mouth activities, and family characteristics.

Fourth and finally, this analysis explains less than a third of the variance in the blood lead levels measured. The remaining unexplained variance may reflect soil lead and air lead measurements that were insufficiently child-specific. The soil lead levels used in the regression reflect only a composite measure for a child's yard. They do not measure the extent of lead in soil with which the child comes in contact during the course of his or her outdoor play. The air lead levels used in the regression reflect area-wide averages and are, at best, only crude estimates of child-specific exposures to breathing-zone air lead levels. Consequently, discussions of specific pathway mechanisms that account for the blood lead levels measured in this study are necessarily limited.

4.4 Comparison of 1983 Montana Blood Lead Data With National Blood Lead Data

National blood lead data collected during the period 1976-1980 showed the mean blood lead level of rural white children, aged 6 months through 5 years, to be 13.5 ± 0.6 ug/dl¹⁰. Given the decreasing trend in average blood lead levels seen during this 4-year period¹¹, when mean blood lead levels of the population aged 6 months to 74 years decreased by about 6 ug/dl, the mean national blood lead level of white rural children in 1983 is probably considerably less than 13.5 ug/dl. Although no 1983 national data are available, Area 3, the comparison area of this study, is likely to represent normal rural conditions accurately in terms of both environmental lead levels and children's blood lead levels. The blood lead levels of children living closest to the smelter are, on the average, twice as high as those of children living in the comparison area, but they do not constitute cause for public health concern.

4.5 Levels of Metals in Blood, Urine, and Hair Samples

Of the various metals analyzed in children's blood, urine, and hair, only mean lead levels in hair differed between the children sampled in Areas 1 and 3. The higher mean hair lead level in Area 1 is consistent with the higher blood lead levels found in that area. Within Area 3, the higher hair

arsenic levels of children who lived in households where someone smoked may reflect a dietary arsenic exposure, although smoke-produced arsenic exposure cannot be ruled out, since tobacco can be contaminated with arsenic through pesticide deposits in the soil.

As for the metals analyzed in adults' urine and hair, no significant between-area differences were found. Within Area 3, the higher levels of cadmium in the urine of adults who were currently smoking are consistent with exposure to cadmium through cigarette smoke.

5.0 References

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6.0 Tables

Table 1

ESA Laboratory Performance in the CDC Blood Lead Proficiency
Testing Program, August-October, 1983

Month	Sample Number*	Target Lead Level (ug/dl)**	Target Range (ug/dl)***	Lead Level Detected by ESA
August	1	14	8-20	13
	2	4	0-10	2
	3	53	45-61	53
September	1	112	95-129	117
	2	69	59-79	70
	3	102	87-117	107
October	1	81	69-93	84
	2	43	37-49	43
	3	4	0-10	3

*Cow blood

**Arithmetic mean of values reported by eight reference laboratories for the sample.

***15% below to 15% above target value. For target values below 40 ug/dl, the target range is 6 ug/dl below to 6 ug/dl above the target value.

Table 2

Mean and Range of Blood Lead Levels

Area	Number of Children Tested	Blood Lead Level (ug/dl)			
		Arithmetic Mean	Geometric Mean	Lowest	Highest
1	98	13.0	11.8	3.0	33.0
2	237	9.4	8.5	1.0	24.0
3	<u>61</u>	6.6	6.0	2.0	17.0
Total	396				

Table 3

ESA Laboratory Performance in the CDC Erythrocyte Protoporphyrin (EP)
Proficiency Testing Program, August-October, 1983.

Month	Sample Number*	Target EP Level (ug/dl)**	Target Range (ug/dl)***	EP Level Detected by ESA
August	1	90	77-103	96
	2	60	51-69	67
	3	161	137-185	174
September	1	150	127-173	156
	2	118	100-136	123
	3	89	76-102	91
October	1	135	115-155	130
	2	197	168-226	194
	3	110	93-127	107

*Spiked outdated human blood from a Blood Bank.

**Arithmetic mean of values reported by nine reference laboratories for the sample.

***15% below to 15% above target value. For target values below 40 ug/dl, the range is 6 ug/dl below to 6 ug/dl above the target value.

Table 4

Mean and Range of Erythrocyte Protoporphyrin (EP) Levels of Children

Area	Number of Children Tested	Erythrocyte Protoporphyrin(ug/dl)			
		Arithmetic Mean	Geometric Mean	Lowest	Highest
1	98	21.9	20.7	11.0	55.0
2	235	20.9	19.7	8.0	77.0
3	61	20.4	19.4	12.0	61.0
Total	394				

Table 5

Mean Blood Lead (BL) and Erythrocyte Protoporphyrin (EP)
Levels by Area and Age.

Area		Age in Years				
		1	2	3	4	5
1	Number	18	22	16	18	24
	BL (ug/dl)	16.2	14.0	10.9	11.6	12.2
	EP (ug/dl)	27.0	24.4	17.0	21.8	19.0
2	Number	39	57	51	55	35
	BL (ug/dl)	8.5	9.7	9.4	10.4	8.7
	EP (ug/dl)	26.8	20.2	20.8*	20.0	16.8**
3	Number	11	16	14	12	8
	BL (ug/dl)	7.2	7.3	6.5	5.7	6.0
	EP (ug/dl)	29.4	18.6	18.3	18.8	18.0

*50 children had EP measurements in this age category in Area 2.

**34 children had EP measurements in this age category in Area 2.

Table 6

Proportions of Children Found To Be Lead Toxic* by Area

Area	Children with Lead Toxicity	Children Without Lead Toxicity
1	1	97
2	0	235
3	0	61

*Defined as blood lead greater than or equal to 25 ug/dl and EP greater than or equal to 35 ug/dl.

Table 7
Lead Levels (ppm) in Soil Samples

Area	Sample Type*	N [@]	Arithmetic Mean	Geometric Mean	Lowest	Highest
1	Comp	71	1,109	720	81	3,414
	Side	71	1,465	796	41	7,964
	Play	55	920	365	3	5,770
	Garden	27	645	539	70	2,038
2	Comp	167	262	217	58	1,252
	Side	93	228	169	3	883
	Play	117	280	121	3	6,030
	Garden	49	220	179	50	599
3	Comp	28	98	86	54	237
	Side	28	120	92	47	500
	Play	20	96	73	28	373
	Garden	5	104	95	58	162

*Comp = Front yard and back yard composited soil

Side = Side yard soil

Play = Play area soil

Garden = Garden soil

@ Sample size

Table 8

Correlation of the Concentrations of Selected Metals Found in
1-and 3- Inch Soil Cores

Element	Sample	Correlation Coefficient	Data Pairs	P
Aluminum	Soil Front	.935	18	LT* .0001
	Soil Side	.836	18	LT .0001
Arsenic	Soil Front	.960	18	LT .0001
	Soil Side	.887	18	LT .0001
Cadmium	Soil Front	.922	18	LT .0001
	Soil Side	.878	18	LT .0001
Copper	Soil Front	.982	18	LT .0001
	Soil Side	.873	18	LT .0001
Lead	Soil Front	.975	18	LT .0001
	Soil Side	.850	18	LT .0001
Titanium	Soil Front	.925	18	LT .0001
	Soil Side	.782	18	LT .0001
Zinc	Soil Front	.983	18	LT .0001
	Soil Side	.929	18	LT .0001

*LT = Less than. Statistically significant correlations are considered to exist when p is less than 0.05.

Table 9

Difference Between the Concentrations of Selected Metals Found in
1- and 3- Inch Soil Cores

Element	Sample@	Mean (ppm)	Mean		Pairs	P*
			Difference			
Aluminum	1" Front	66,185	-783.90	18	.134	
	3" Front	66,970				
	1" Side	66,104	-1,321.30	18	.097	
	3" Side	67,425				
Arsenic	1" Front	137.2	5.61	18	.387	
	3" Front	132.6				
	1" Side	155.1	29.35	18	.341	
	3" Side	125.8				
Cadmium	1" Front	31.04	-1.14	18	.566	
	3" Front	32.18				
	1" Side	34.42	5.89	18	.159	
	3" Side	28.52				
Copper	1" Front	110.26	-18.69	18	.031	
	3" Front	128.94				
	1" Side	145.68	27.93	18	.510	
	3" Side	117.76				

(Table 9, continued)

Lead	1" Front	677.22			
	3" Front	633.19	44.02	18	.366
	1" Side	792.59			
	3" Side	512.27	280.32	18	.204
Titanium	1" Front	2,768.67			
	3" Front	2,899.11	-130.44	18	.010
	1" Side	2,771.35			
	3" Side	2,912.23	-140.88	18	.231
Zinc	1" Front	513.64			
	3" Front	562.26	-48.62	18	.101
	1" Side	539.01			
	3" Side	513.06	25.96	18	.706

@1" Front = 1-inch core sample collected from front yard.

3" Front = 3-inch core sample collected from front yard.

1" Side = 1-inch core sample collected from side yard.

3" Side = 3-inch core sample collected from side yard.

*Statistically significant differences are considered to exist when p is less than 0.05.

Table 10

Heavy Metal Contents of "Largely Uncontaminated"
Soil Samples Collected Worldwide⁹

Metal	Sample Size	Concentration Range (ppm)	Mean Concentration (ppm)
Aluminum	1,770	700 - 203,000	66,500
Arsenic	1,193	0 - 194	11.3
Cadmium	1,642	<0.005 - 10	0.62
Copper	7,819	1 - 389	25.8
Lead	4,970	<1 - 888	29.2
Titanium	2,192	<60 - 34,000	5,091
Zinc	7,402	1.5 - 2,000	59.8

Table 11

Lead Levels in Dust Samples Collected from Household Vacuum
Cleaner Bags and From Floor Wipes

Area	Sample Type	N*	Arithmetic Mean	Geometric Mean	Lowest	Highest
1	Vacuum**	54	2,186	1,588	240	18,361
	Wipe@	54	0.32	1.02	0.02	2.35
2	Vacuum	99	687	561	119	2,651
	Wipe	158	0.12	0.04	0.02	1.74
3	Vacuum	26	449	380	80	1,351
	Wipe	35	0.07	0.06	0.02	1.00

*Sample size.

**Lead levels are given in parts per million.

@Lead levels are given in units of 10^{-7} grams per square centimeter.

Table 12

Number of Floor-Wipe Samples That Had Concentrations
Below ICP/AAS Detection Limits

Metal	No. Samples	No. at or Below Detection	Percent Below Detection
Al	355	21	5.9
AS	366	36	9.8
Cd	354	222	62.7
Cu	354	53	15.0
Pb	354	141	39.8
Ti	354	47	13.3
Zn	<u>354</u>	30	8.5
Total	2,497		

Table 13

Lead Levels ($\mu\text{g}/\text{cm}^2$ of filter) in Dust Samples Collected
From Vacuuming One Square Meter of Carpet

Area	N*	Geometric Mean	Arithmetic Mean	Lowest	Highest
1	28	0.4358	0.9119	0.015	6.6715
3	22	0.0311	0.0588	0.015	0.2083

*Sample Size.

Table 14

Lead Levels (ppm) in Garden Vegetables

Area	N ^a	Arithmetic Mean	Geometric Mean *	Lowest	Highest
1#	69	31	23	2	140
2	122	34	17	1	339
3	18	8	4	0.2	36

^aSample size.

*Based on a national survey¹², expected mean lead levels are 0.11 ppm in root vegetables, such as carrots and beets, and .050 ppm in leafy vegetables, such as lettuce.

#One vegetable sample in Area 1 had a lead value of 6,380 ppm. This outlier was excluded in calculating the statistics listed in this table.

Table 15

Lead Paint XRF Calibration Readings

<u>Calibration Readings</u>	<u>Number of Households With Readings</u>	<u>Number of Children Who Had Household Readings</u>
Missing	22	24
Outside Acceptable Range	194	234
Within Acceptable Range	102	138
Total	318	396

Table 16

Lead Levels Detected in Household Painted Surfaces (mg/cm²)

Area	Lead Level	Interpretation	Number of Surfaces
1	Less than 0.7	Negative	351
	0.7 to 2.9	Low	38
	3.0 to 5.9	Moderate	13
	6.0 or greater	High	18
2	Less than 0.7	Negative	685
	0.7 to 2.9	Low	22
	3.0 to 5.9	Moderate	1
	6.0 or greater	High	2
3	Less than 0.7	Negative	224
	0.7 to 2.9	Low	17
	3.0 to 5.9	Moderate	4
	6.0 or greater	High	6

Table 17

Ambient Air Quality Summary: Mean Lead Levels*

Area	Sampling Site	July	August	September	July - September
1	Firehall	4.981 (7)	4.76 (10)	4.65 (8)	4.79 (25)
	Hadfield	1.73 (3)	2.92 (11)	3.49 (10)	3.01 (24)
	Hastie	2.64 (8)	3.09 (8)	3.08 (8)	3.01 (24)
	Dartman	2.09 (6)	3.33 (10)	3.14 (9)	2.96 (25)
2	Schneider	1.80 (3)	2.06 (9)	1.99 (7)	2.00 (19)
	Dudley	0.34 (3)	0.27 (10)	0.28 (9)	0.28 (22)
	South	0.87 (6)	0.83 (11)	0.69 (10)	0.79 (27)
3	Townsend	0.07 (1)	0.25 (10)	0.18 (10)	0.21 (21)

*All units are micrograms per cubic meter of air adjusted to 760 mm of mercury and 25°C. Numbers of samples collected appear in parentheses.

Table 18

Levels of Metals (ng/ml) Detected in Handwash Samples

	Arsenic	Cadmium	Copper	Lead	Titanium	Zinc
All Areas						
Mean	2.07	5.05	44.65	45.96	4.19	113.16
Std. Dev.	5.69	6.34	124.27	100.97	4.53	124.58
N	371	373	373	373	373	373
Area 1						
Mean	4.77	5.68	9.29	90.84	4.43	120.99
Std. Dev.	10.21	5.74	238.80	182.06	4.97	113.90
N	97	95	95	95	95	95
Area 2						
Mean	1.29	4.89	25.82	34.15	3.94	112.29
Std. Dev.	2.10	6.52	35.43	42.42	4.10	130.69
N	221	224	224	224	224	224
Area 3						
Mean	0.384	4.68	26.68	15.99	4.80	103.01
Std. Dev.	0.33	6.60	37.20	8.90	5.37	117.62
N	53	54	54	54	54	54

Table 19

Number of Handwash Samples That Had Concentrations
Below ICP/AAS Detection Limits

<u>Metal</u>	<u>No. Samples</u>	<u>No. at or Below Det.</u>	<u>Percent Below Det.</u>
Al	647	85	13.1
As	643	319	49.6
Cd	647	503	77.7
Cu	647	128	19.8
Pb	647	329	50.9
Si	149	111	74.5
Ti	647	551	85.2
Zn	<u>647</u>	53	8.2
Total Samples	4,674		

Table 20

Correlation Between Original and Duplicate
Samples Taken From Individual Households

<u>Element</u>	<u>Sample</u>	<u>Correlation Coefficient</u>	<u>Data Pairs</u>	<u>P</u>
Arsenic	Floor Wipe	.910	28	LT* .0001
	Soil Front	.961	18	LT .0001
	Soil Side	.975	18	LT .0001
Lead	Floor Wipe	.951	28	LT .0001
	Soil Front	.985	18	LT .0001
	Soil Side	.985	18	LT .0001

*LT = Less Than

Table 21

Difference Between the Concentrations of Selected Metals in
Original and Duplicate Samples:
Paired T-Test

Element	Sample	Mean	Mean Difference	Data Pairs	P*
Aluminum	Floor Wipe	112.5	-6.1	28	.557
	Duplicate	118.6			
	Soil, Front	66,597	682.3	18	.644
	Duplicate	65,401			
Arsenic	Floor Wipe	1.37	0.04	28	.746
	Duplicate	1.33			
	Soil, Front	140.6	4.08	18	.535
	Duplicate	136.6			
	Soil, Side	146.1	-7.33	19	.630
	Duplicate	153.5			
Cadmium	Floor Wipe	1.30	-1.09	28	.437
	Duplicate	2.40			
	Soil, Front	30.6	0.12	19	.952
	Duplicate	30.5			
Lead	Floor Wipe	15.9	1.86	28	.201
	Duplicate	14.1			
	Soil, Front	727.3	52.56	18	.214
	Duplicate	674.8			
	Soil, Side	748.0	-43.88	19	.595
	Duplicate	791.9			

(Table 21, continued)

Element	Sample	Mean	Mean Difference	Data Pairs	P*
Titanium	Floor Wipe	6.02			
	Duplicate	6.45	-0.43	28	.620
	Soil, Front	2,783			
	Duplicate	2,765	18.7	18	.670
	Soil, Side	2,831			
	Duplicate	2,764	67.3	19	.468
Zinc	Floor Wipe	25.9			
	Duplicate	24.4	1.44	28	.482
	Soil, Front	551.0			
	Duplicate	518.5	32.47	18	.541
	Soil, Side	566.2			
	Duplicate	545.1	21.14	19	.50

*Probability that the original and duplicate sample concentrations are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 22

Correlations Among Log-Transformed Lead Levels in
Soil and Dust Samples, All Areas

Front yard & Back yard Composite Soil Sample (Composite)	Side Yard Soil Sample (Side)	Play Area Soil Sample (Play)	Vacuum- Bag Dust Grab Sample (Dust)	Garden Soil Sample (Garden)
Composite	1.0*	0.78	0.50	0.76
0**	0.0001	0.0001	0.0001	0.0001
266@	192	192	171	81
Side	1.0	0.50	0.72	0.72
	0	0.0001	0.0001	0.0001
	192	137	131	60
Play		1.0	0.46	0.59
		0	0.0001	0.0001
		192	126	71
Dust			1.0	0.74
			0	0.0001
			179	53
Garden				1.0
				0
				81

*Pearson correlation coefficient.

**Significance probability of the correlation.

@Number of samples.

Table 23

Mean Blood Lead Levels (ug/dl)
According To Lead Content of Front yard and Back yard
Composited Soil Samples*

Area	Composite Soil Lead (ppm)			
	Less Than 250	251-750	751-1,500	1,501-3,500
1	21.0 (3)	10.8 (29)	12.8 (21)	16.8 (28)
2	9.3 (15)	9.6 (74)	9.7 (6)	--
3	6.4 (20)	--	--	--

*Numbers of children sampled are in parentheses.

Table 24

Mean EP Levels (ug/dl) According To Lead Content
of Front and Back yard Composited Soil Samples*

Area	Composite Soil Lead (ppm)			
	Less Than 250	251-750	750-1,500	1,501-3,500
1	27.7 (3)	19.5 (29)	25.7 (21)	21.9 (28)
2	19.8 (14)	23.0 (73)	15.5 (6)	
3	20.0 (20)			

*Numbers of children sampled appear in parenthesis.

Table 25

Mean Ages (Years) of Children Whose Mean Blood Lead Levels
Are Displayed in Table 14*

Area	Composite Soil Lead (ppm)			
	Less Than 250	251-750	751-1,500	1,501-3,500
1	2.5 (3)	4.1 (29)	3.2 (21)	3.4 (28)
2	3.3 (15)	3.4 (74)	3.6 (6)	--
3	3.4 (20)	--	--	--

*Numbers of children sampled are in parentheses.

Table 26

Number of Children in Each Area
According to Front yard and Back yard Grass Coverage

Area	Percentage of Yard Covered by Grass	
	0-50%	50-100%
1	4	88
2	16	207
3	2	55

Chi-square = 1.64, p = 0.44

Table 27

Mean Blood Lead Levels (ug/dl)
According To Front yard and Back yard Grass Coverage*

Area	Percentage of Yard Covered by Grass			
	0 - 25%	25 - 50%	50 - 75%	75 - 100%
1	12.5 (2)	16.0 (2)	15.5 (4)	12.9 (84)
2	11.6 (8)	9.8 (8)	8.4 (16)	9.3 (191)
3	--	5.0 (2)	11.0 (3)	6.5 (52)

*Numbers of children sampled are in parentheses.

Table 28

Mean Blood Lead Levels (ug/dl)
According To Lead Content of Side Yard Soil Samples*

Area	Side-Yard Soil Lead (ppm)			
	Less Than 250	251-750	751-1,500	1,501-8,000
1	10.0 (17)	10.9 (30)	12.5 (24)	17.4 (24)
2	9.2 (91)	9.9 (34)	11.0 (3)	--
3	6.8 (36)	5.8 (5)	--	--

*Numbers of children sampled are in parentheses.

Table 29

Mean Blood Lead Levels (ug/dl)
According To Lead Content of Vacuum-Bag Dust Grab Samples*

Vacuum-Bag Dust Lead (ppm)						
Area	Less Than 500	501-1,000	1,001-1,500	1,501-2,000	2,001-2,500	2,501-8,000
1 [#]	11.5 (2)	7.7 (18)	11.8 (11)	12.6 (18)	17.8 (5)	17.1 (16)
2	8.0 (63)	9.5 (52)	10.6 (10)	13.0 (6)	18.0 (1)	13.5 (4)
3	6.7 (27)	5.6 (10)	6.0 (1)	--	--	--

*Numbers of children sampled are in parentheses.

#Two children in Area were associated with a vacuum-bag dust lead level of 18,360 ppm; the mean blood lead level of these two children was 18.0 ug/dl.

Table 30

Number of Households From Which Vacuum-Bag
Dust Samples Were Collected According To Lead Content of
Vacuum-Bag Dust Grab Samples

House-Dust Lead Category (ppm)						
Area	Less Than 500	501-1,000	1,001-1,500	1,501-2,000	2,001-2,500	2,501-8,000
1	2	13	10	13	4	11
2	43	40	8	4	1	2
3	17	8	1	-	-	-

Table 31

Mean Blood Lead Levels (ug/dl) According To
Lead Content of Floor Dust Wipe Samples*

Area	Floor Dust Wipe Lead (ppm)			
	Less Than 0.08	0.081-0.150	0.151-0.400	0.401-3.00
1	14.8 (19)	10.7 (14)	14.5 (14)	15.8 (13)
2	10.5 (21)	9.6 (37)	11.8 (26)	18.0 (4)
3	7.4 (9)	9.0 (1)	6.0 (1)	8.0 (1)

*Numbers of children sampled are in parentheses.

Table 32

Children in Households With and Without Active Lead Hobbyists

Area	Number of Children in Households Where at Least One Lead-Related Hobby Was Practiced	Number of Children in Households Where No Lead-Related Hobby Was Practiced	Total
1	54	44	98
2	118	119	237
3	<u>35</u>	<u>26</u>	<u>61</u>
Total	207	189	396

Chi-square = 1.537; p = 0.46

Table 33

Mean Blood Lead Levels (ug/dl) According To the
Presence or Absence of Lead-Related Hobbies*

Area	Children in Households Where at Least One Lead-Related Hobby Was Practiced	Children in Households Where No Lead-Related Hobby Was Practiced	t**	p@
1	13.4 (6.2)	12.6 (5.5)	0.64	0.52
2	9.7 (3.9)	9.2 (4.5)	1.69	0.09#
3	7.2 (3.2)	5.8 (2.5)	2.09	0.04

*Standard deviations appear in parentheses.

**Student's t statistic of the log-transformed blood lead levels.

@Probability that the two log-transformed mean blood lead levels are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

#Corrections have been made for unequal variations.

Table 34

Mean EP Levels (ug/dl) According To the
Presence or Absence of Lead-Related Hobbies*

Area	Children in Households Where at Least One Lead-Related Hobby Was Practiced	Children in Households Where No Lead-Related Hobby Was Practiced	t**	p@
1	21.8 (8.8)	21.9 (7.9)	-0.16	0.87
2	19.7 (7.2)	22.1 (8.9)	-2.64	0.009
3	20.1 (6.0)	20.9 (9.9)	-0.02	0.98

*Standard deviations are in parentheses.

**Student's t statistic of the log-transformed EP levels.

@Probability that the two log-transformed mean EP levels are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 35

Children Living in Households With and Without Storm Windows

Area	Number of Children Living in Houses With Storm Windows	Number of Children Living in Houses Without Storm Windows	Total
1	75	20	95
2	182	51	233
3	<u>50</u>	<u>10</u>	<u>60</u>
Total	307	81	388

Chi-square = 0.79, p = 0.67

Table 36

Mean Blood Lead Levels (ug/dl) According To Household Storm Window Use*

Area	Children in Households With Storm Windows	Children in Households Without Storm Windows	t**	p@
1	13.5 (6.3)	11.6 (3.8)	0.80	0.42
2	9.4 (4.1)	9.5 (4.8)	0.52	0.60
3	7.0 (3.0)	5.0 (2.4)	2.36	0.02

*Standard deviations are in parentheses.

**Student's t statistic of the log-transformed blood lead levels.

@Probability that the two log-transformed mean blood lead levels are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 37
Mean EP Levels (ug/dl) According To Household
Storm Window Usage*

Area	Children in Houses With Storm Windows	Children in Houses Without Storm Windows	t**	p@
1	21.4 (8.5)	23.4 (7.9)	-1.21	0.23
2	20.5 (7.7)	22.2 (9.8)	1.12	0.26
3	20.7 (8.4)	19.4 (4.6)	0.24	0.81

*Standard deviations are in parentheses.

**Student's t statistic of the log-transformed EP levels.

@Probability that the two log-transformed mean EP levels are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 38

Mean House Dust Lead Levels (ppm) According To
Household Storm Window Use

Area	Houses With Storm Windows	Houses Without Storm Windows	t**	p@
1	2,292	1,928	0.16	0.87
2	691	699	-0.33	0.74
3	477	333	1.36	0.19

**Student's t statistic of the log-transformed dust lead levels.

@Probability that the two log-transformed mean house dust lead levels are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 39

Children's Frequencies of Eating Neighborhood-Grown
Fruits or Vegetables

Area	Number of Children Who Frequently Ate Neighborhood Produce	Number of Children Who Did Not Frequently Eat Neighborhood Produce	Total
1	44	54	98
2	105	130	235
3	<u>33</u>	<u>28</u>	<u>61</u>
Total	182	212	394

Chi-square = 1.816, p = 0.40.

Table 40

Mean Blood Lead Levels (ug/dl) According To
Frequency of Eating Neighborhood-Grown
Fruits or Vegetables*

Area	Children Who Frequently Ate Neighborhood Produce	Children Who Did Not Frequently Eat Neighborhood Produce	t**	p@
1	12.9 (5.2)	13.2 (6.4)	0.10	0.92
2	9.4 (4.2)	9.5 (4.2)	-0.24	0.81
3	6.4 (3.0)	6.8 (3.0)	-0.24	0.81

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 41

Mean EP Levels (ug/dl) According To
Frequency of Eating Neighborhood-Grown
Fruits or Vegetables*

Area	Children Who Frequently Ate Neighborhood Produce	Children Who Did Not Frequently Eat Neighborhood Produce	t**	p@
1	22.0 (9.2)	21.8 (7.6)	-0.14	0.89
2	19.2 (6.8)	22.4 (8.9)	-3.34	0.001
3	19.9 (6.4)	21.1 (9.4)	-0.54	0.59

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 42

Children's Use of Vitamins, Minerals, or
Other Dietary Supplements

Area	Number of Children Taking Supplements	Number of Children Not Taking Supplements	Total
1	67	31	98
2	136	101	237
3	<u>43</u>	<u>18</u>	<u>61</u>
Total	246	150	396

Chi-square = 5.70, p = 0.058

Table 43

Mean Blood Lead Levels (ug/dl) According To
Use of Vitamins, Minerals, or
Other Dietary Supplements*

Area	Children Taking Supplements	Children Not Taking Supplements	t**	p@
1	12.6 (6.0)	13.9 (5.6)	-1.28	0.20
2	9.1 (3.9)	9.9 (4.5)	-1.43	0.15
3	6.5 (2.8)	6.9 (3.3)	-0.16	0.87

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 44

Mean EP Levels (ug/dl) According To
Use of Vitamins, Minerals, or
Other Dietary Supplements*

Area	Children Taking Supplements	Children Not Taking Supplements	t**	p@
1	21.6 (8.6)	22.4 (7.9)	-0.62	0.54
2	20.3 (7.0)	21.7 (9.5)	-0.89#	0.38
3	19.2 (5.8)	23.5 (11.1)	-1.92	0.06

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

#Corrections have been made for unequal variances.

Table 45

Children's Use of Grassy and
Nongrassy Play Surfaces

Area	Number of Children Using Grassy Play Surfaces	Number of Children Not Using Nongrassy Play Surfaces	Total
1	27	71	98
2	56	181	237
3	<u>17</u>	<u>44</u>	<u>61</u>
Total	100	296	396

Chi-square = 0.827, p = 0.66

Table 46

Mean Blood Lead Levels (ug/dl) According To
Children's Use of Grassy and Nongrassy
Play Surfaces*

Area	Children Who Played on Grassy Surfaces	Children Who Played on Nongrassy Surfaces	t**	p@
1	16.4 (6.6)	11.8 (5.0)	3.47	0.0008
2	8.8 (3.9)	9.6 (4.3)	0.97	0.34
3	5.9 (2.3)	6.9 (3.2)	0.99	0.32

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 47

Mean EP Levels (ug/dl) According To
Children's Use of Grassy and Nongrassy
Play Surfaces*

Area	Children Who Played on Grassy Surfaces	Children Who Played on Nongrassy Surfaces	t**	p@
1	25.3 (10.6)	20.6 (6.9)	2.62	0.010
2	20.6(5.7)	21.0 (8.8)	0.15	0.88
3	22.9 (10.9)	19.5 (6.2)	1.5	0.14

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 48

Children in Households With and Without
Household Members Who Smoked

Area	Number of Children in Households Where Someone Smoked	Number of Children in Households Where Nobody Smoked	Total
1	44	54	98
2	123	112	235
3	<u>22</u>	<u>39</u>	<u>61</u>
Total	189	205	394

Chi-square = 5.63, p = 0.06

Table 49

Mean Blood Lead Levels (ug/dl) According To
the Presence or Absence of a
Household Member Who Smoked*

Area	Children in Households Where Someone Smoked	Children in Households Where Nobody Smoked	t**	p@
1	14.3 (5.9)	12.0 (5.7)	2.17	0.032
2	10.4 (4.6)	8.4 (3.4)	3.32	0.001
3	6.0 (2.2)	6.9 (3.3)	0.67	0.51

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 50

Mean EP Levels (ug/dl) According To
the Presence or Absence of a
Household Member Who Smoked*

Area	Children in Households	Children in Households	t**	p@
	Where Someone Smoked	Where Nobody Smoked		
1	22.4 (8.3)	21.5 (8.5)	0.71	0.48
2	21.1 (8.3)	20.8 (8.1)	0.40	0.68
3	18.8 (10.2)	21.4 (6.2)	-2.03	0.047

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 51

Children's Habits of
Taking Food Outside

Area	Number of	Number of	Total
	Children Who Often Took Food Outside	Children Who Did Not Often Take Food Outside	
1	49	47	96
2	99	128	227
3	<u>25</u>	<u>33</u>	<u>58</u>
Total	173	208	381

Chi-square = 1.65, p = 0.44.

Table 52

Mean Blood Lead Levels (ug/dl) According To
Children's Habits of
Taking Food Outside*

Area	Children Who Often Took Food Outside	Children Who Did Not Often Take Food Outside	t**	p@
1	13.7 (7.0)	12.5 (4.5)	0.23#	0.81
2	9.7 (4.1)	9.2 (4.3)	1.03	0.30
3	6.6 (3.1)	6.4 (2.9)	0.26	0.80

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

#Corrections have been made for unequal variances.

Table 53

Mean EP Levels (ug/dl) According To
Children's Habits of
Taking Food Outside*

Area	Children Who Often Took Food Outside	Children Who Did Not Often Take Food Outside	t**	p@
1	21.6 (8.6)	22.0 (8.1)	-0.30	0.77
2	20.3 (8.1)	20.9 (8.2)	-0.71	0.48
3	19.2 (5.6)	21.3 (9.3)	-0.88	0.38

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 54

Children's Habits of Using a Pacifier,
Sucking a Thumb, or Chewing Fingernails

Area	Number of Children Who Had These Oral Habits	Number of Children Who Did Not Have These Oral Habits	Total
1	43	55	98
2	92	145	237
3	<u>21</u>	<u>40</u>	<u>61</u>
Total	156	240	396

Chi-square = 1.49, p = 0.48.

Table 55

Mean Blood Lead Levels (ug/dl) According To
Children's Habits of Using a Pacifier,
Sucking a Thumb, or Chewing Fingernails*

Area	Children Who Had These Oral Habits	Children Who Did Not Have These Oral Habits	t**	p@
1	13.3 (6.0)	12.8 (5.8)	0.37	0.71
2	9.8 (4.2)	9.2 (4.2)	1.16	0.24
3	6.6 (3.7)	6.6 (2.6)	-0.28	0.78

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed blood lead levels.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 56

Mean EP Levels (ug/dl) According To
Children's Habits of Using a Pacifier,
Sucking a Thumb, or Chewing Fingernails*

Area	Children Who Had These Oral Habits	Children Who Did Not Have These Oral Habits	t**	p@
1	22.0 (7.9)	21.7 (8.8)	0.41	0.68
2	20.8 (7.9)	21.0 (8.4)	-0.20	0.84
3	20.9 (11.4)	20.2 (5.3)	-0.32#	0.75

*Standard deviations are in parentheses.

**Student's t statistic for the log-transformed EP levels.

@Probability that the log-transformed mean EP levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

#Corrections have been made for unequal variances.

Table 57

Mean Blood Lead Levels (ug/dl) According To the Absence
or Presence of Lead Paint in the Household*

Area	Children in Households Without Lead Paint	Children in Households With Lead Paint	t**	p@
1	13.9 (13)	15.3 (29)	-0.64	0.50
2	8.4 (54)	8.5 (17)	-0.05	0.94
3	4.8 (13)	6.6 (12)	-1.73	0.02

*Number of children sampled are in parentheses.

**Student's t statistic.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

Table 58

Mean Blood Lead Levels (ug/dl) According To the Absence or Presence
of Chipping or Peeling Lead Paint in the Household*

Area	Children in Households Without Lead Paint or With Intact Lead Paint Surfaces	Children in Households With Lead Paint and Peeling Lead Paint Surfaces	t**	p@
1	13.7 (15)	15.6 (27)	-1.28	0.21
2	8.6 (51) ⁺	8.5 (19)	-0.24	0.81
3	4.8 (13)	6.6 (12)	-1.73	0.02

*Number of children sampled are in parentheses.

**Student's t statistic.

@Probability that the log-transformed mean blood lead levels for the two groups within one area are essentially the same. Statistically significant differences are considered to exist when p is less than 0.05.

⁺Fewer cases than in Table 51 due to missing data on surface chipping or peeling for three children.

Table 59

Correlations of Blood Lead Levels in Sibling Pairs

Maximum Age Difference Between Siblings	<u>Sibling 1</u> Mean Blood Lead Level (ug/dl) S..D.* N**			<u>Sibling 2</u> Mean Blood Lead Level (ug/dl) S.D.* N**			Correlation
1 year	9.2	4.3	10	8.2	4.3	10	0.62
2 years	10.2	6.3	54	9.1	5.2	54	0.75
3 years	10.0	5.5	92	9.0	4.6	92	0.70

*Standard deviation.

**Sample size.

Table 60

Principal Component Analysis of Children's
Mouthing Behavior Variables
(304 Observations, 7 Variables)

	Eigenvectors		
	MOUTH 1	MOUTH 2	MOUTH 3
Eigenvalue	1.02934	0.84308	0.46843
Difference	0.18626	0.37465	0.21232
Proportion	0.32271	0.26432	0.14686
Variable Weights*			
EATSNOWT	0.03943	-.00212	-.13094
ORAL	-.01634	-.04381	-.14119
CPUFDT	0.00823	-.09518	-.09796
CORIFU	0.20532	0.78667	-.57062
CORIoT	0.97212	-.16910	0.12881
CORIPA	0.02538	0.57278	0.74705
CORISW	-.10140	0.11440	0.23030

*Variables defined as follows:

EATSNOWT - does the child eat snow?

ORAL - does the child suck thumb or fingers, suck a pacifier, or chew nails?

CPUFDT - does the child often take some food or a bottle with him/her outside to play?

CORIFU - does the child often put mouth on furniture or window sill?

CORIoT - does the child put things other than food in mouth?

CORIPA - have you ever seen child put paint chips in mouth?

CORISW - does child swallow things other than food?

Table 61

Final Set of Multiple Regression Analyses

Dependent Variable = ln (blood lead)							
Model*	n	Backward Stepwise			MAXR Stepwise		
		r-square	b**	p-value	r-square	b	p-value
1. Soil	358	0.170	0.2058	0.0001	0.170	0.2058	0.0001
2. Dust	246	0.247	0.2888	0.0001	0.247	0.2888	0.0001
3. Air+@	161	0.184	0.1420	0.0001	0.184	0.1420	0.0001
4. (3)+soil	356	0.198	soil 0.1430	0.0001	0.198	soil 0.1430	0.0001
			air 0.0649	0.0083		air 0.0649	0.0083
5. (3)+dust	246	0.276	dust 0.2353	0.0001	0.276	dust 0.2353	0.0001
			air 0.0556	0.0452		air 0.0556	0.0452
6. (3)+soil, dust	234	0.270	soil 0.0952	0.0208	0.270	soil 0.0952	0.0208
			dust 0.2015	0.0001		dust 0.2015	0.0001
7. (3)+soil, dust, areal area2	234	0.282	dust 0.2232	0.0001	0.282	dust 0.2232	0.0001
			air 0.3304	0.0040		areal 0.3067	0.0064
			areal -0.8398	0.0110		area2 0.2478	0.0040

*independent variables used in model construction

**the unstandardized regression coefficient for air lead, for the natural log transformations of soil lead or dust lead, or for area, as identified in the table.

@PBHOBby, STORMWIN, AGES, AGES_SQ, INCOME, MOUTH1*, MOUTH2*, CPHOR, CORIFL, SMOKET, VITAMINT, and WASH. (* Variables used to construct these principal component vectors were: EATSNOWT, ORAL, CPUFDT, CORIFU, CORIOT, CORIPA, and CORISW.)

#the variables in the most predictive model from the preceding step, i.e., SMOKET and AIR.

Table 62

Definitions of Variables Used in Final Set of
Multiple Regression Analyses (Table 61)

Variable	Definition	Type
Soil	Soil lead level	Continuous
Dust	Dust lead level	Continuous
Air	Air lead level	Continuous
Area1	Home not in Area 2 or in Area 3	Categorical
Area2	Home not in Area 1 or in Area 3	Categorical
PBHOBBY	Absence or presence of lead hobby in home	Categorical
STORMWIN	Absence or presence of storm windows	Categorical
AGES	Child's age	Continuous
AGES-SQ	Child's age squared	Continuous
INCOME	Family Income	Categorical
MOUTH1	Eigenvector (see Table 60)	Continuous
MOUTH2	" " " "	Continuous
CPOHR	Daily number of hours of outdoor play in the neighborhood	Continuous
CORIFL	Daily number of hours of indoor play on the floor	Continuous
SMOKET	Absence or presence of smoker in home	Categorical
VITAMINT	Use or nonuse of vitamins, minerals, or other dietary supplements	Categorical

Table 63

Testing for the Influence of Hand-to-Mouth Activity

<u>Dependent Variable = ln (blood lead)</u>							
		Backward Stepwise			MAXR Stepwise		
<u>Model@</u>	<u>n</u>	<u>r-square</u>	<u>b</u>	<u>p-value</u>	<u>r-square</u>	<u>b</u>	<u>p-value</u>
LN(DUST)	234	0.282		0.0001	0.282		0.0001
AIR			0.2232	0.0001		0.2232	0.0001
SMOKET			0.3304	0.0040		0.3304	0.0040
AREA1			0.1184	0.0439		0.1184	0.0439
AGEMARK*			-.8398	0.0110		-.8398	0.0110
LN(DUST)	246	0.293		0.0001	0.293		0.0001
AIR			0.2296	0.0001		0.2296	0.0001
SMOKET			0.3178	0.0047		0.3178	0.0047
AREA1			0.1194	0.0377		0.1194	0.0377
AGEMARK**			-.7812	0.0158		-.7812	0.0158

@Independent variables used in model construction.

*AGEMARK is an interaction term between (1) a categorical variable whose value 1 denotes age less than or equal to 2 years and whose value 2 denotes age greater than 2 years and (2) the natural log transformations of soil lead levels. Although included in the model construction, AGEMARK did not appear as a significant contributor to the dependent variable, i.e., the natural log transformations of blood lead.

**AGEMARK is an interaction term between (1) a categorical variable whose value 1 denotes age less than or equal to 2 years and whose value 2 denotes age greater than 2 years and (2) the natural log transformations of dust lead levels. Although included in the model construction, AGEMARK did not appear as a significant contributor to the dependent variable, i.e., as the natural log transformation of blood lead.

Table 64

Final Multiple Regression Results For Predicting Children's
Blood Lead LevelsLn Blood Lead (n = 234; $r^2 = 0.28$)

Variable*	Parameter Estimate	F statistic	p [#]
ALPHA	0.3328		
LN (DUST)	0.2232	27.62	0.0001
AIR	0.3304	8.46	0.004
AREA 1	-0.8398	6.57	0.011
SMOKET	0.1184	4.11	0.044

*ALPHA = Y-axis intercept.

LN(DUST) = Natural logarithm of lead level found in grab sample for household vacuum cleaner.

AIR = Area-specific ambient air lead level.

AREA 1 = Home is in Area 1.

SMOKET = Someone in household smokes.

[#]Significance level. Variables are considered to be statistically significant contributors to log-transformed blood lead when p is less than 0.05.

Table 65

Mean Levels of Metals in Blood, Urine, and Hair Samples From Children by Area*

Area	Blood Cadmium (ng/ml)	Urine Lead (ng/ml)**	Urine Arsenic (ng/ml)**	Hair Lead (ug/g)	Hair Cadmium (ug/g)	Hair Arsenic (ug/g)
1	0.50 (16)	2.6 (17)	9.8 (17)	15.5 (13)	0.83 (13)	772 (12)
3	0.42 (9)	1.0 (16)	7.0 (16)	6.5 (16)	0.62 (16)	258 (8)
T test Probability†	0.44	0.17@	0.60@	0.02@	0.28	0.09@

*Sample sizes are in parentheses.

**Standardized to 10 mg creatinine/dl.

†Probability that the mean levels are essentially the same in both areas.
Statistically significant differences are considered to exist when p is less
than 0.05.

@Corrections have been made for unequal variances.

Table 66

Mean Levels of Metals in Children's Blood, Urine, and Hair Samples
According To Household Storm-window Use*

Area	Sample**	Children Living in Households Without Storm-Windows	Children Living in Households With Storm-Windows	p#
1	BCD	0.46 (7)	0.53 (9)	0.55
	UPB	2.16 (6)	.88 (11)	0.74
	UAS	1.5 2 (6)	14.3 (11)	0.11@
	HPB	19.2 (4)	13.9 (9)	0.62
	HCD	0.74 (4)	0.87 (9)	0.71
	HAS	688 (4)	813 (8)	0.82
3	BCD	0.0 (1)	0.48 (8)	A
	UPB	2.49 (4)	0.55 (12)	0.35@
	UAS	11.3 (4)	5.6 (12)	0.18
	HPB	4.2 (3)	7.0 (13)	0.27
	HCD	0.31 (3)	0.70 (13)	0.20
	HAS	0.0 (1)	295 (7)	A

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UPB = Urine lead (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (mg/ml), standardized to 10 mg.

HPB = Hair lead (ug/g).

HCD = Hair cadmium (ug/g).

HAS = Hair arsenic (ug/g).

#Probability that the mean levels are essentially the same in both as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

A All values are the same for one class level; no t test performed.

Table 67

Mean Levels of Metals in Children's Blood, Urine, and Hair Samples
According to Play Surface Type*

Area	Sample**	Children Who Played Mainly on Grassy Surfaces	Children Who Played Mainly on Nongrassy Surfaces	p#
1	BCD	0.63 (3)	0.48 (13)	0.45
	UPB	0.69 (3)	3.04 (14)	0.08@
	UAS	11.1 (3)	9.6 (14)	0.91
	HPB	21.2 (3)	13.8 (10)	0.60@
	HCD	0.88 (3)	0.82 (10)	0.88
	HAS	512 (3)	858 (9)	0.58
3	BCD	0.60 (1)	0.40 (8)	A
	HPB	0.22 (3)	1.22 (13)	0.42
	UAS	4.49 (3)	7.61 (13)	0.52
	HPB	6.36 (3)	6.56 (13)	0.94
	HCD	0.49 (3)	0.67 (13)	0.46
	HAS	399 (1)	238 (7)	A

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UPB = Urine lead (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

HPB = Hair lead (ug/g).

HCD = Hair cadmium (ug/g).

HAS = Hair arsenic (ug/g).

#Probability that the mean levels are essentially the same as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

A All values are the same for one class level; no t test performed.

Table 68

Mean Levels of Metals in Children's Blood, Urine, and Hair Samples
According To Absence and Presence of Household Member Who Smoked*

Area	Samples**	Children Who Lived With No Smoker	Children Who Lived With One or More Smokers	p#
1	BCD	0.41 (9)	0.61 (7)	0.10
	UPB	1.85 (10)	3.73 (7)	0.38
	UAS	11.8 (10)	7.0 (7)	0.65
	HPB	13.2 (6)	17.5 (7)	0.52
	HCD	0.65 (6)	0.99 (7)	0.29
	HAS	384 (6)	1159 (9)	0.15@
3	BCD	0.37 (7)	0.60 (2)	0.047@
	UPB	1.15 (12)	0.70 (4)	0.69
	UAS	6.67 (12)	8.09 (4)	0.74
	HPB	7.25 (13)	3.36 (3)	0.12
	HCD	0.70 (13)	0.28 (3)	0.15
	HAS	122 (6)	665 (2)	0.03

*Sample sizes are in parentheses.

**BCD = Blood Cadmium (ng/ml).

UPB = Urine lead (mg/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

HPB = Hair lead (ug/g).

HCD = Hair cadmium (ug/g).

HAS = Hair arsenic (ug/g).

#Probability that the mean levels are essentially the same as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

Table 69

Mean Levels of Metals in Blood and Urine
Samples from Adults by Area*

Area	Blood Cadmium (ng/ml)	Urine Cadmium (ng/ml)**	Urine Arsenic (ng/ml)**
1	0.93 (15)	0.56 (16)	4.44 (16)
3	1.05 (6)	0.32 (16)	5.60 (16)
T Test Probability#	0.74	0.26 [@]	0.65

*Sample sizes are in parentheses.

**Standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

Table 70

Area-Specific Mean Adult Ages and Lengths of Continuous
Residence in Homes Where Interviews Were Conducted*

Area	Mean Age (Years)	Mean Length of Residence (Years)
1	61.6 (17)	31.6 (17)
3	62.3 (16)	24.0 (16)
T Test Probability #	0.90	0.31

*Sample sizes are in parentheses

#Probability that the means are essentially the same in both areas. Statistically significant differences are considered to exist when p is less than 0.05.

Table 71

Mean Levels of Metals in Adult Blood and Urine Samples According To
Their or Their Spouse's History of Having Worked in a Lead-Related Industry*

Area	Sample**	Adults With Lead-Job History	Adults With No Lead-Job History	p#
1	BCD	0.77 (9)	1.17 (6)	0.34@
	UCD	0.51 (9)	0.63 (7)	0.77
	UAS	6.26 (9)	2.09 (7)	0.24
3	BCD	-- (1)	1.05 (6)	A
	UCD	0.23 (1)	0.32 (15)	A
	UAS	0.0 (1)	5.97 (15)	A

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UCD = Urine cadmium (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas, as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

A All values are the same for one class level; no t test performed.

Table 72

Mean Levels of Metals in Adult Blood and Urine Samples According To
Yard-Working Habits*

Area	Sample**	Adults Who Worked in Their Yards and/ or Their Neighbor's Yards	Adults Who Did No Yard Work	p#
1	BCD	0.94 (14)	0.70 (1)	A
	UCD	0.59 (15)	0.15 (1)	A
	UAS	4.57 (15)	2.45 (1)	A
3	BCD	1.47 (3)	0.63 (3)	0.37
	UCD	0.37 (11)	0.22 (5)	0.51
	UAS	6.04 (11)	4.62 (5)	0.74

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UCD = Urine cadmium (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas, as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

A All values are the same for one class level; no t test performed.

Table 73

Mean Levels of Metals in Adult Blood and Urine Samples According To
Habits of Eating Neighborhood-Grown Fruits and Vegetables*

Area	Sample**	Adults Who Ate Neighborhood Produce	Adults Who Did Not Eat Neighborhood Produce	P#
1	BCD	0.93 (15)	-- (0)	A
	UCD	0.60 (15)	0.06 (1)	A
	UAS	4.66 (15)	1.11 (1)	A
3	BCD	1.27 (3)	0.83 (3)	0.66
	UCD	0.38 (9)	0.24 (7)	0.49
	UAS	4.66 (9)	8.01 (7)	0.28

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UCD = Urine cadmium (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas,
as analyzed by the Student's t test. Statistically significant
differences are considered to exist when p is less than 0.05.

Table 74

Mean Levels of Metals in Adult Blood and Urine Samples
According To Habits of Eating Fish Caught Locally*

Area	Sample**	Adults Who Ate Locally Caught Fish	Adults Who Did Not Eat Locally Caught Fish	p#
1	BCD	0.83 (7)	1.01 (8)	0.57@
	UCD	0.38 (7)	0.71 (9)	0.39
	UAS	6.65 (7)	2.71 (9)	0.32@
3	BCD	0.70 (2)	1.22 (4)	0.61
	UCD	0.09 (2)	0.37 (13)	0.40
	UAS	6.99 (2)	5.81 (13)	0.85

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UCD = Urine cadmium (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas, as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

Table 75

Mean Levels of Metals in Adult Blood and Urine Samples According
To Current Cigarette-Smoking Habits*

Area	Sample**	Adults Who Currently Smoked	Adults Who Did Not Currently Smoke	P#
1	BCD	1.53 (3)	0.92 (5)	0.49@
	UCD	1.30 (3)	0.29 (5)	0.32@
	UAS	0.82 (3)	1.98 (5)	0.61
3	BCD	1.50 (3)	0.70 (2)	0.50
	UCD	0.63 (7)	0.09 (5)	0.02@
	UAS	7.76 (7)	2.98 (5)	0.34

*Sample sizes are in parentheses.

**BCD = Blood cadmium (ng/ml).

UCD = Urine cadmium (ng/ml), standardized to 10 mg creatinine/dl.

UAS = Urine arsenic (ng/ml), standardized to 10 mg creatinine/dl.

#Probability that the mean levels are essentially the same in both areas, as analyzed by the Student's t test. Statistically significant differences are considered to exist when p is less than 0.05.

@Corrections have been made for unequal variances.

APPENDIX 1
STATE OF MONTANA

Department of Health and Environmental Sciences

NEWS RELEASE

DATE: July 29, 1983

CONTACT: Hal Robbins
Air Quality Bureau
449-3454

HELENA---A public meeting to explain the scope and aims of the "Superfund" study of East Helena children's exposure to lead will be held at 7 p.m. Tuesday, August 2 in the East Helena fire hall.

A canvass began last week to identify households in East Helena, and the control area in northeast Helena, that contain children from one to five years of age. All families in such households will then be asked to participate in the study.

Qualified medical and technical personnel from the Lewis and Clark City/County Health Department will be doing the biologic and environmental sampling necessary to obtain data necessary to complete the study.

Blood samples of children will be taken, as well as random samples of urine, hair and stools from a select number of children and urine samples from some adults. Children's hands will be dipped into a weak, vinegar-like solution of nitric acid.

Lead content will also be checked in paint samples from household walls and baseboards, household dust, yard and garden soil, and some garden vegetables.

Analysis of samples will begin immediately, and should be completed by November 1. Homeowners will be notified of any indications that samples contain lead in excess of established safety standards.

According to Hal Robbins, State Department of Health and Environmental Sciences, participation in the census and study is strictly voluntary, and any information given to interviewers will be held in strict confidence.

Robbins emphasized that the department considers this an extremely important health-related study, as the last one undertaken in the area was done seven years ago.

The project has been reviewed and approved by the Centers for Disease Control; and by the Environmental Protection Agency. The \$385,000 cost of the study comes from the EPA's Superfund program, which was set up to clean up existing hazardous and toxic wastes, and for special research projects such as the East Helena study.

APPENDIX 2

FROM THE DIRECTOR
MONTANA DEPARTMENT OF HEALTH AND ENVIRONMENTAL SCIENCES

You may have read in the newspaper--or heard on the radio or television--about a study planned this summer which will determine children's exposure to lead for families living in the Helena Valley. All families in the East Helena area and families in the northeast part of Helena will be contacted to locate preschool children during the next few weeks.

A Department of Health census worker will call on you to ask whether you have young children, their ages, and when you and your children will be home this summer. They may also ask whether you have a garden or not, your employment, and length of East Helena area residency. Your information will be used to locate families who will again be visited in July and August, 1983, to gather blood, possibly urine, and environmental samples. All information you give to the interviewer will be held in strict confidence. Any information released by the State Department of Health and Environmental Sciences will be used only for statistical purposes in a manner in which no information about you as an individual can be identified.

The Department considers this to be an extremely important health-related study and desires to sample all children in the 1 through 5 year old group. Though your participation is voluntary, the real benefits may come to your children. Your cooperation will be appreciated both in answering the census questions and later with the collection of samples.

On the other side of this letter are the answers to questions you may have about this study.

Thank you for your cooperation.

Sincerely,

John Drynan, M.D.

What is this study all about? The Department will conduct this study of all children in selected areas to locate those who might have more lead levels in their bodies than those established by the Centers for Disease Control (CDC) of Atlanta, GA. If such children are identified, they will be encouraged to seek medical assistance, and the sources of lead in their environments will be determined, so clean-up measures can follow.

Who is involved? Montana State Health Department, CDC, the Environmental Protection Agency (EPA), and the City-County Health Department will all be involved in this same manner with this project. ASARCO and the East Helena school system are also helping with the study.

How was my family selected? You live in one of the three geographical study areas. The first area includes those who live within one-mile radius of the ASARCO lead smelter and essentially includes the City of East Helena. Families between 1 and 2.25 miles radius of the smelter are in the second area. Those living in the northeast part of Helena, north of Prospect, east of Harris, and west of the Interstate, constitute the third (control) area. Yours is one of about 375 families participating in the study.

Who is paying for the project? CDC and EPA are providing funds for this work.

How will samples be collected? East Helena area children will be asked to visit a clinic where medical people will draw blood samples. Thereafter teams will visit homes where preschool children live to collect blood, from those who didn't participate in the clinics. In some instances urine may also be collected. House dusts, yard and garden soils and some vegetables will be sampled to characterize the nearby environment. A questionnaire will be filled out for each child.

How often will I be visited? Most families will be visited twice--once during the census interview and again when a team visits your home. Additional visits may be requested of some families.

What about the test results of my children and my residence? You will be given the results either by letter or during a visit by a Department representative. Explanation of the results will come from your physician and or Department personnel.

APPENDIX 3

Block # _____

Area _____

File _____

Eligible C. _____

Eligible A. _____

I.D. / / / / / / / / / /

Letter _____

Montana Department of Health
and Environmental Sciences

Date 1 (T) _____

Map _____

Date 2 (T) _____

Vacant _____

Date 3 (T) _____

QUESTIONNAIRE -83(1)

No Contact / / / /

I. IDENTIFICATION (All residences) / /

1. Name _____ Person Interviewed _____

2. Address _____ Phone _____

3. Mailing address (If different) _____

4. Years at this Address _____ Head of Household _____ Spouse _____

5. Years in East Helena Study Area _____

6. Age _____

7. Work in E. Helena industry Y/N _____ Yrs: _____ Y/N _____ Yrs. _____

8. Farm/ranch near E.H. Y/N _____ Yrs. _____ Y/N _____ Yrs. _____

9. Do you have a garden Y/N _____ Yrs. _____ Y/N _____ Yrs. _____

10. How many families live at this address? _____

II. QUESTIONS FOR ALL FAMILIES WITH CHILDREN AGES 1 THROUGH 5 / /

1. Names of all children Normally at residence	Birthdate			Sex		Household Member* Y N	Relationship
	M	D	Y	M	F		
A. _____	/	/	/	/	/	/	_____
B. _____	/	/	/	/	/	/	_____
C. _____	/	/	/	/	/	/	_____
D. _____	/	/	/	/	/	/	_____
E. _____	/	/	/	/	/	/	_____

2. Will you be on vacation between July 18 and August 19? Y/N _____
If yes, when will you be away? _____3. Is it best to visit you and your children -morning _____
(during the period July 18-August 19) -afternoon _____
-evening _____

III. CONTACTS: Date _____ Date _____ Date _____ Comments _____

Personal _____

Telephone _____

*Is this usual place or residence _____

APPENDIX 4

MORTON

Ref.
Area
Block
Eligible-C
Eligible-A

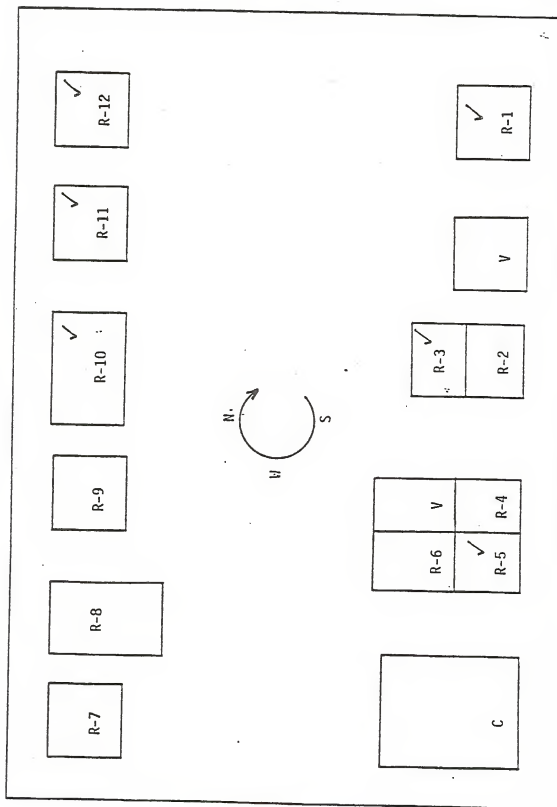
E-H

27

✓

SE.

RIGGS



MAIN STREET

(Begin)
SE.

HARRISON

KEY: R = Residential, V = Vacant, C = Commercial

APPENDIX 5

Participant Consent - General

I understand that the Montana Department of Health and Environmental Sciences, with the assistance of the Center for Disease Control is conducting a study to determine the possible health effects of lead exposure on pre-school age children living in East Helena, its vicinity, and in a control area.

I understand that there will be three parts to the study:

A. Interview -- Collection of information concerning:

- (1) Health history, habits and activities of children in my home from 1 through 5 years of age;
- (2) Occupations of adults in my home,
- (3) Types of cooking utensils used in my home, and
- (4) Hobbies of any family member involving lead.

B. Environmental Testing

- (1) Collection of dust samples within my home,
- (2) Collection of soil and vegetable samples in the yard of my home (leaving small holes),
- (3) Examination of walls at my home for presence of lead in paint. (This will be done by placing an x-ray fluorescent instrument against the wall. The procedure will not damage nor alter the appearance of the wall.)
- (4) Washing of child's hands to measure lead which may enter the mouth. The wash solution, a solution similar to vinegar, will be washed from the hands after the sampling.

C. Examination of Blood samples

- (1) A blood sample, approximately 2-3 ml. will be taken from a vein in the arm of each child in my household ages 1 through 5 years to be tested for indicators of lead toxicity. (There should be no problems

associated with collecting the blood sample, other than slight, temporary discomfort and the possibility of a small bruise at the site where the needle enters the skin, which will disappear in a few days.)

I voluntarily agree to take part in this study and consent to having my child/children participate. I understand that my/my children's participation involves: (1) being interviewed regarding the topics described above, (2) contributing a sample of blood, urine and hand dust, (3) allowing soil, dust and vegetable samples to be taken from my residence, and (4) allowing examination of the paint in my home. I have been assured that personal identifying information will be kept in confidence by DHES and neither I nor any member of my family will be identified by name in published reports of the results of this study. I also understand that I may decline to answer specific questions as I see fit and that I am free to withdraw my/our child's participation in the study at any time. I understand that I will be informed in writing of the results of these tests at the completion of the study, unless additional followup is indicated, in which case, I will be notified immediately. I understand that if I have further questions concerning the study, information can be obtained by contacting the Air Quality Bureau at 449-3454.

Participant

Signed: _____
Parent/Guardian

Street _____

City/State _____

Interviewer

Signed _____

Date _____

1 copy to participant
1 copy to investigator

Participant Consent - Adults

I understand that the Montana Department of Health and Environmental Sciences, (DHES) with the assistance of the Centers for Disease Control (CDC) is conducting a study to determine the possible health effects of lead and cadmium exposure on people living in Esat Helena, its vicinity and in a control area. I understand that there will be two parts to the study:

A. Environmental Testing

- (1) Collection of dust samples within my home
- (2) Collection of soil and vegetable samples in the yard of my home (leaving small holes)

B. Examination of blood and urine samples

- (1) A blood sample, approximately 2-3 ml. will be taken from a vein in my arm to be tested for indicators of lead toxicity. (There should be no problems associated with collecting the blood sample, other than slight, temporary discomfort and the possibility of a small bruise at the site where the needle enters the skin, which will disappear in a few days).
- (2) A urine specimen will be collected for cadmium analysis.

I voluntarily agree to take part in this study. I understand that my participation involves: (1) Contributing a sample of blood and urine, and (2) allowing soil, dust and vegetable samples to be taken from my residence. Any leftover biologic specimens may be retained by CDC. I have been assured that personal identifying information will be kept in confidence by DHES and CDC and neither I nor any member of my family will be identified by name in published reports of the results of this study. The information shared with CDC will be protected under the Federal Privacy Act.

I also understand that I may decline to answer specific questions as I see fit and that I am free to withdraw my participation in the study at any time. I understand that I will be informed in writing of the results of these tests at the completion of the study, unless additional follow-up is indicated, in which case I will be notified immediately. I understand that if I have further questions concerning the study, information can be obtained by contacting the Air Quality Bureau at 449-3454.

Participant

Signed: _____

Street _____

City/State _____

Interviewer

Signed _____

Date _____

1 Copy to participant

1 copy to investigator

INTERVIEWING PROCEDURES

1. Complete necessary information before the interview. Label consent form and interview form. Label each page with ID #.
2. Introduce yourself and your team members. Briefly explain everyone's function in the study.
3. Read over the Participant Consent form with the volunteer, including a brief explanation of all three parts of the study.
 - A. Interview
 - B. Environmental Testing
 - C. Biological Samples
4. Discuss any questions and ensure confidentiality.
5. Obtain volunteer signature on consent form and witness signature in writing.
6. Proceed with questioning until finished.
7. Complete necessary information at end of interview.

(1-7) I.D. Number /
 (Block) (House)

EAST HELENA, MONTANA
CHILD QUESTIONNAIRE
CHILDHOOD LEAD EXPOSURE

1. Complete before interview:

A. Address: _____
 No. Street Apt. #

(8-13) B. Date of interview / /
Mo. Day Yr.

(14-15) C. Interviewer No.

(16-19) D. Time interview began: _____ (Convert to military time)

MILITARY TIMETABLE

0100 AM	0700 AM	1300 PM	1900 PM
0200 AM	0800 AM	1400 PM	2000 PM
0300 AM	0900 AM	1500 PM	2100 PM
0400 AM	1000 AM	1600 PM	2200 PM
0500 AM	1100 AM	1700 PM	2300 PM
0600 AM	1200 N	1800 PM	2400 M

(20) 2. I would like to talk to the parent or legal guardian of the Children who live in this house, preferably the one who can tell us about how the other family members, especially the child spends its time or younger children spend their time. Is that person you?

1 = Yes 2 = No

(If answer is "no", ask who that person is and if you can come back later to talk to him/her. Discontinue interview until you are talking to who can tell you how the child/children spend their time.)

This report is authorized by law (PL 96-510, Sect. 104 (b)). While your response is voluntary, your cooperation is appreciated.

I.D. # _____/_____

Page 2

3. What is your name? _____
First Middle Last

(21) 4. How long has this family been living at this address:

- 1 = Less than 1 month
- 2 = 1 month or more but less than 2 months
- 3 = 2 to 3 months
- 4 = More than 3 months but less than 6 months
- 5 = 6 months to 1 year
- 6 = More than 1 year but less than 5 years
- 7 = 5 years or more
- 9 = Don't know or Unknown

IF THREE MONTHS OR LESS, TERMINATE INTERVIEW

5. Total number of persons living in household including any baby, small children, and persons who usually live here but who are away now, traveling, on vacation, in a hospital, or somewhere else.
(Include yourself)

(ENTER ANSWER ONLY WHEN YOU ARE SURE OF THE TOTAL NUMBER)

(22-23) Total Number: _____

RESPONSES IN TABLE 1 ON NEXT PAGE

6. A. What is the full name of youngest person living in household?

B. Circle code for sex; ask if necessary

C. On what date was he/she born?

Who is the next youngest? (Proceed up in age.)

(Repeat A,B,C for all members of the household.)

D. Ask as appropriate for all persons 16 and older:
What is (his/her/your) occupation—that is, what does (he/she, do you) do?

HOUSEHOLD ROSTER
TABLE 1

	Person	A Name	B Sex	C Date of Birth	D Occupation
(24-25)	01	F _____	M 1	MO. _____ (27-28)	
		M _____	(26)	DAY _____ (29-30)	
		L _____	F 2	YR. _____ (31-32)	
				AGE _____ (33-34)	
(35-36)	02	F _____	M 1	Mo. _____ (38-39)	
		M _____	(37)	Day _____ (40-41)	
		L _____	F 2	Yr. _____ (42-43)	
				Age _____ (44-45)	
(46-47)	03	F _____	M 1	Mo. _____ (49-50)	
		M _____	(48)	Day _____ (51-52)	
		L _____	F 2	Yr. _____ (53-54)	
				Age _____ (55-56)	
(57-58)	04	F _____	M 1	Mo. _____ (60-61)	
		M _____	(59)	Day _____ (62-63)	
		L _____	F 2	Yr. _____ (64-65)	
				Age _____ (66-67)	
(68-69)	05	F _____	M 1	Mo. _____ (71-72)	
		M _____	(70)	Day _____ (73-74)	
		L _____	F 2	Yr. _____ (75-76)	
				Age _____ (77-78)	

	A	B	C	D
Person	Name	Sex	Date of Birth	Occupation
(79-80) 06	F _____	M 1	Mo. _____ (82-83)	
	M _____	(81)	Day _____ (84-85)	
	L _____	F 2	Yr. _____ (86-87)	
			Age _____ (88-89)	
(90-91) 07	F _____	M 1	Mo. _____ (93-94)	
	M _____	(92)	Day _____ (95-96)	
	L _____	F 2	Yr. _____ (97-98)	
			Age _____ (99-100)	
(101-102) 08	F _____	M 1	Mo. _____ (104-105)	
	M _____	(103)	Day _____ (106-107)	
	L _____	F 2	Yr. _____ (108-109)	
			Age _____ (110-111)	
(112-113) 09	F _____	M 1	Mo. _____ (115-116)	
	M _____	(114)	Day _____ (117-118)	
	L _____	F 2	Yr. _____ (119-120)	
			Age _____ (121-122)	
(123-124) 10	F _____	M 1	Mo. _____ (126-127)	
	M _____	(125)	Day _____ (128-129)	
	L _____	F 2	Yr. _____ (130-131)	
			Age _____ (132-133)	

(134) 7. Are you the head of the household?

- 1 = Yes (Head)
- 2 = Yes (Co-Head)
- 3 = No

(135-136) 8. A. Who is the head of the household?
(Put person "NUMBER" according to table 1.)

(137) B. What is the highest grade or year of regular school that
(NAME OF HEAD) finished and got credit for?

- 1 = Graduate work
- 2 = 4 - year college degree
- 3 = Some college
- 4 = High School graduate
- 5 = Some High School
- 6 = 7th or 8th grade
- 7 = Less than seventh grade
- 9 = Don't know or unknown

(138) C. Which of the statements below comes closest to the
total family income for this family before taxes in 1982?

- 1 = Under \$5,000
- 2 = \$5,000 or more but less than \$10,000
- 3 = \$10,000 or more but less than \$15,000
- 4 = \$15,000 or more but less than \$20,000
- 5 = \$20,000 or more but less than \$25,000
- 6 = \$25,000 or more
- 7 = Refused
- 9 = Don't know or unknown

(139) 9. I'm going to read you a list of different kinds of jobs that
expose people to lead dust or vapors. Have you or any member of
this household worked in one or more of these jobs during the
last 3 months?

- 1 = Yes
- 2 = No
- 9 = Unknown

If yes, circle which ones --

LEAD-ZINC RELATED OCCUPATION CODES

- | | |
|--|------------------------------------|
| (140-141) 01 = Lead smelter worker | (152-153) 07 = Paint-pigment, zinc |
| (142-143) 02 = Foundry Worker | copper Worker |
| (144-145) 03 = Oil Refinery Worker | (154-155) 08 = Plumber |
| (146-147) 04 = Painter | (156-157) 09 = Glass Worker |
| (148-149) 05 = Battery Mfg. Plant Worker | (158-159) 10 = Other Lead-Related |
| (150-151) 06 = Chemical Plant Worker | Industry Worker |

Answer following questions in Table 2 for each person who has worked in a lead-zinc related occupation

- A. What is the name of the place where (you/he/she) work(s)?
- B. How long have you (has he/she) worked there?
- C. Do(es) (you/he/she) change out of (your/his/her) work clothes and leave them at work?
- D. Do(es) (you/he/she) shower at work before coming home?
- E. What is (your/his/her) job title?

LEAD-RELATED JOBS
TABLE 2

(160-161)	Person No. _____	A. _____ (Name of Workplace)	
		B. Time at workplace Months: _____ (162-164)	C. Change Clothes 1 = Yes 2 = No 9 = Don't know (165)
(166-167)	Occupation Code _____	D. Shower 1 = Yes 2 = No 9 = Don't know or Unknown (168)	E. _____ (Job Title)
(169-170)	Person No. _____	A. _____ (Name of Workplace)	
		B. Time at workplace Months: _____ (171-173)	C. Change Clothes 1 = Yes 2 = No 9 = Don't know (174)
(175-176)	Occupation Code _____	D. Shower 1 = Yes 2 = No 9 = Don't know or Unknown (177)	E. _____ (Job Title)

(178-179) Person No. _____

A. _____
(Name of Workplace)

B. Time at workplace

Months:

(180-182)

C. Change Clothes

1 = Yes

2 = No

9 = Don't know

(184-185) Occupation
Code _____

D. Shower

1 = Yes

2 = No

9 = Don't know or Unknown

E. _____
(Job Title)

(187-188) Person No. _____

A. _____
(Name of Workplace)

B. Time at workplace

Months:

(189-191)

C. Change clothes

1 = Yes

2 = No

9 = Don't know

(193-194) Occupation
Code _____

D. Shower

1 = Yes

2 = No

9 = Don't know or Unknown

E. _____
(Job Title)

(196-197) Person No. _____

A. _____
(Name of Workplace)

B. Time at workplace

Months:

(198-200)

C. Change clothes

1 = Yes

2 = No

9 = Don't know

(202-203) Occupation
Code _____

D. Shower

1 = Yes

2 = No

9 = Don't know or Unknown

E. _____
(Job Title)

	Child No.	10A Where Spends Time During Day 1 2 3 4 5	10B Hours Away	10C Address away from home
(217)	3	(218-220)	(221-222)	
				Number Street
				City State Zip Code

	Child No.	10A Where Spends Time During Day 1 2 3 4 5	10B Hours Away	10C Address away from home
(223)	4	(224-226)	(227-228)	
				Number Street
				City State Zip Code

	Child No.	10A Where Spends Time During Day 1 2 3 4 5	10B Hours Away	10C Address away from home
(229)	5	(230-232)	(233-234)	
				Number Street
				City State Zip Code

	Child No.	10A Where Spends Time During Day 1 2 3 4 5	10B Hours Away	10C Address away from home
(235)	6	(236-238)	(239-240)	
				Number Street
				City State Zip Code

11. Where does (he/she) usually play when outdoors around this home?

1 = In back yard
2 = In front yard

3 = Does not play outdoors
8 = Other (Specify)

TABLE 4
ACCEPT MULTIPLE ANSWERS

	Child No.		USUALLY PLAYS				SPECIFY OTHER
(241)	1	(242 - 245)	1	2	3	8	_____
(246)	2	(247 - 250)	1	2	3	8	_____
(251)	3	(252 - 255)	1	2	3	8	_____
(256)	4	(257 - 260)	1	2	3	8	_____
(261)	5	(262 - 265)	1	2	3	8	_____
(266)	6	(267 - 270)	1	2	3	8	_____

12. Is the ground there mainly grassy, concrete/asphalt, plain dirt or soil, just a sandbox, or what?

1 = Grassy
2 = Concrete/Asphalt
3 = Dirt/Soil

4 = Sandbox
7 = Other (Specify)
8 = Not applicable

TABLE 5
ACCEPT MULTIPLE ANSWERS
(Circle answers).

	CHILD NO.		GROUND MAINLY						Specify Other
(271)	1	(272 - 277)	1	2	3	4	7	8	_____
(278)	2	(279 - 284)	1	2	3	4	7	8	_____
(285)	3	(286 - 291)	1	2	3	4	7	8	_____
(292)	4	(293 - 298)	1	2	3	4	7	8	_____
(299)	5	(300 - 305)	1	2	3	4	7	8	_____
(306)	6	(307 - 312)	1	2	3	4	7	8	_____

I.D. # _____/_____

Page 11

13. About how many hours each day does (he/she) usually spend playing outdoors in this neighborhood?
14. Does (he/she) often take some food or a bottle with (him/her) outside to play?

1 = Yes
2 = No

8 = Not applicable
9 = Don't know or Unknown

TABLE 6

	CHILD No.		14 HOURS	15 FOOD/BOTTLE
(313)	1	(314-315)	_____	(316) 1 2 8 9
(317)	2	(318-319)	_____	(320) 1 2 8 9
(321)	3	(322-323)	_____	(324) 1 2 8 9
(325)	4	(326-327)	_____	(328) 1 2 8 9
(329)	5	(330-331)	_____	(332) 1 2 8 9
(333)	6	(334-335)	_____	(336) 1 2 8 9

15. Does (CHILD No. __) usually play alone, with other children, or mostly with adults?

1 = Alone
2 = Other children
3 = Adults

TABLE 7

Child No.				
(337)	1	(338 - 340)	1	2 3
(341)	2	(342 - 344)	1	2 3
(345)	3	(346 - 348)	1	2 3
(349)	4	(350 - 352)	1	2 3
(353)	5	(354 - 356)	1	2 3
(357)	6	(358 - 360)	1	2 3

ENTER ANSWERS TO QUESTIONS 16 - 18 IN TABLE 8 BELOW

16. Are (his/her) hand or face usually washed before eating?
 17. Are (his/her) hands or face usually washed before going to sleep?
 18. Are (his/her) hands or face usually washed after making mud pies,
 or playing with dirt or sand?

TABLE 8

1 = Yes, 2 = No, 8 = Not applicable, 9 = Don't know or Unknown

Child No.		BEFORE EATS	BEFORE SLEEP	AFTER PLAY
(361)	1	(362 - 364) 1 2 8 9	1 2 8 9	1 2 8 9
(365)	2	(366 - 368) 1 2 8 9	1 2 8 9	1 2 8 9
(369)	3	(370 - 372) 1 2 8 9	1 2 8 9	1 2 8 9
(373)	4	(374 - 376) 1 2 8 9	1 2 8 9	1 2 8 9
(377)	5	(378 - 380) 1 2 8 9	1 2 8 9	1 2 8 9
(381)	6	(382 - 384) 1 2 8 9	1 2 8 9	1 2 8 9

ENTER ANSWERS TO QUESTIONS 19 - 22 IN TABLE 9 BELOW

19. Has (CHILD NO.) used a pacifier often in the last 3 months?
20. Does (he/she) often suck (his/her) thumb or fingers?
21. Does (he/she) sometimes chew on (his/her) fingernails?
22. A. Does (he/she) have a favorite blanket or stuffed toy?

IF YES ASK B AND C. OTHERWISE SKIP TO QUESTION 23.

B. Does (he/she) carry this around during the day?

C. Does (he/she) often put this in (his/her) mouth?

TABLE 9

1 = Yes, 2 = No, 8 = Not applicable, 9 = Don't know or Unknown
(Circle correct number)

Child No.	19 PACIFIER	20 SUCK THUMB	21 CHEW NAILS	22A FAVORITE	22B CARRY	22C TOY IN MOUTH
(385-391) 1	1 2 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9
(392-398) 2	1 2 9 -	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9
(399-405) 3	1 2 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9
(406-412) 4	1 2 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9
(413-419) 5	1 2 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9
(420-426) 6	1 2 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9	1 2 8 9

ENTER ANSWERS TO QUESTIONS 23 - 27 IN TABLE 10 BELOW.

23. How many hours during the day do you think (he/she) usually spend playing on the floor when indoors in this home.
24. Does (he/she) often put (his/her) mouth on furniture or on the window sill?
25. Many children put some things other than food into their mouths. Would you say that (CHILD NO. __) does this a lot, just once in a while or almost never?
26. Have you ever seen (CHILD NO. __) put paint chips in his/her mouth?
27. Sometimes children swallow things other than food. Would you say that (Child No. __) swallows things other than food a lot, just once in a while, or almost never?

TABLE 10

1 = A lot, 2 = Just once in a while,
3 = Almost never, 9 = Don't know or Unknown

	CHILD NO.	23 HOURS	24 FURNITURE	25 THINGS IN MOUTH	26 PAINT CHIPS	27 SWALLOW
(427-433)	1	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9
(434-440)	2	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9
(441-447)	3	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9
(448-454)	4	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9
(455-461)	5	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9
(462-468)	6	_____	1 2 9	1 2 3 9	1 2 3	1 2 3 9

*27a. Specify things swallowed:

Child No.	Item(s) swallowed
_____	_____
_____	_____
_____	_____

I.D. # _____/_____

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RECORD ANSWERS TO QUESTION 28 IN TABLE 11 BELOW

28. A. During the last 3 months has (he/she) been taking any vitamins?

B. During the last 3 months has (he/she) been taking any minerals?

C. Has (he/she) taken any other kind of dietary supplement in the last 3 months?

TABLE 11

1 = Yes, 2 = No, 9 = Don't know or Unknown

CHILD NO.	28A VITAMINS	28B MINERALS	28C SUPPLEMENT
(469)	1	(470) 1 2 9	(471) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____
(473)	2	(474) 1 2 9	(475) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____
(477)	3	(478) 1 2 9	(479) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____
(481)	4	(482) 1 2 9	(483) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____
(485)	5	(486) 1 2 9	(487) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____
(489)	6	(490) 1 2 9	(491) 1 2 9
	NAME _____	NAME _____	NAME _____
	NAME _____	NAME _____	NAME _____

ENTER ANSWERS TO QUESTIONS 29 AND 30 IN TABLE 12 BELOW

29. A. Has (Child No.) participated in any kind of lead poisoning screening program within the last 12 months?

1 = Yes, 2 = No, 9 = Don't know or Unknown

IF "NO" SKIP TO QUESTION 30.

B. What were the results of that screening; were they normal or high?

1 = Normal, 2 = High, 9 = Don't Know or Unknown

30. Circle code for race of child. IF NECESSARY, ASK: What race is (Child No.)?

1 = White, Non-Hispanic

2 = White, Hispanic

3 = Black, Non-Hispanic

4 = Black, Hispanic

5 = Asian or Pacific Islander

6 = American Indian or

Native

7 = Refused ...

9 = Unknown

TABLE 12
(Circle correct number)

Child No.	30A SCREENED	30B RESULTS	31 RACE
(493-496) 1	1 2 9	1 2 9	1 2 3 4 5 6 7 9
(497-500) 2	1 2 9	1 2 9	1 2 3 4 5 6 7 9
(501-504) 3	1 2 9	1 2 9	1 2 3 4 5 6 7 9
(505-508) 4	1 2 9	1 2 9	1 2 3 4 5 6 7 9
(509-512) 5	1 2 9	1 2 9	1 2 3 4 5 6 7 9
(513-516) 6	1 2 9	1 2 9	1 2 3 4 5 6 7 9

BE SURE QUESTIONS 10-30 HAVE BEEN ASKED FOR EACH CHILD UNDER 6 IN THE HOUSEHOLD

31. Thinking about the kinds of hobbies that people might have, or work or activities that people might do around the home, within the past 3 months has any member of this household often:

HOBBIE CODES

1 = Yes, No = 2, 9 = Unknown

- | | | | | |
|-------|---|---|---|--|
| (517) | 1 | 2 | 9 | A. Painted pictures with artists paint? |
| (518) | 1 | 2 | 9 | B. Painted parts of the house or furniture in the house? |
| (519) | 1 | 2 | 9 | C. Worked with stained glass? |
| (520) | 1 | 2 | 9 | D. Cast lead into fishing sinkers, bullets or anything else? |
| (521) | 1 | 2 | 9 | E. Worked with soldering in electronics? |
| (522) | 1 | 2 | 9 | F. Worked on soldering pipes? |
| (523) | 1 | 2 | 9 | G. Made pottery at home? |

1 = Yes, 2 = No, 9 = Don't know or Unknown

- | | | | | |
|-------|---|---|---|--|
| (524) | 1 | 2 | 9 | 32. Does/do the child/children frequently eat fruits or vegetables grown anywhere close by in this neighborhood? |
| (525) | 1 | 2 | 9 | 33. Does your family have a garden? |
| (526) | 1 | 2 | 9 | 34. Has soil been hauled in and placed on your garden? |
| (527) | 1 | 2 | 9 | 35. When food is served, is it ever served in homemade or imported clay pottery or ceramic dishes? |
| (528) | 1 | 2 | 9 | 36. And when food is stored or put away for a while, is it sometimes stored in the original can after being opened? Give example if necessary: e.g. juice can. |
| (529) | 1 | 2 | 9 | 37. Does/do the child/children sometimes eat snow in the wintertime? |
| (530) | 1 | 2 | 9 | 38. Does anyone in this household smoke? |
| (531) | 1 | 2 | 9 | 39. Does your home have storm windows? |
| | | | | 40. What is the name of the medical doctor who generally examines or treats your child/children? |
| (532) | 1 | 2 | 9 | 1. _____ |
| | | | | 2. Have no family doctor |
| | | | | 9. Don't know |

I.D. # _____/_____

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- (533) 1 2 3 49. A. Thank you very much for answering these questions. There may be a few things we talked about that I need to clarify. May I telephone you if I need to?

1 = Yes, 2 = No, 3 = No Phone

IF YES, ASK:

- B. What is your telephone number? (or that of nearby neighbor or relative?)
_____/_____/_____

- (534) 1 2 9 50. Year house built
1 = Before 1955
2 = After 1955
9 = Don't know

II. COMPLETE AFTER INTERVIEW:

- A. Time Interview Ended
(Military Time)

(535 - 538)

0100 AM	0700 AM	1300 PM	1900 PM
0200 AM	0800 AM	1400 PM	2000 PM
0300 AM	0900 AM	1500 PM	2100 PM
0400 AM	1000 AM	1600 PM	2200 PM
0500 AM	1100 AM	1700 PM	2300 PM
0600 AM	1200 N	1800 PM	2400 M

- B. Type of Housing Structure

- (539) _____ 1. Constructed of:
1 = Frame 3 = Concrete Block
2 = Brick 4 = Combination
5 = Other (Describe)

- (540) _____ 2. Structure is:
1 = Sgl., 2 = 2-Family, 3 = 3-or more Family

(541-542) _____ Number of Units

(1-7) I.D.# _____ / _____
(Block) (House)

ADULT QUESTIONNAIRE
CHILDHOOD LEAD EXPOSURE
EAST HELENA, MONTANA

DEMOGRAPHIC

1. Subject's name _____
(Last First Middle)
- (8-13) 2. Subject's date of birth: Mo. _____ Day _____ Yr. _____
- (14) 3. Subject's sex: _____ 1 = Male 2 = Female
- (15) 4. Subject's race: _____
1 = White (not hispanic)
2 = Black (not hispanic)
3 = Hispanic
4 = American Indian or Alaskan native
5 = Asian or Pacific Islander
9 = Unknown
5. Subject's telephone: _____ / _____ / _____
(Area)

6. RESIDENCE:

- A. List all places you have lived in the past 30 years,
starting with the most recent -
(Note to interviewer: Fill in last two
digits of year; e.g. 82 for 1982.)

	FROM	TO	ADDRESS
(16-19)	---	---	_____
(20-23)	---	---	_____
(24-27)	---	---	_____
(28-31)	---	---	_____
(32-35)	---	---	_____
(36-39)	---	---	_____
(40-43)	---	---	_____
(44-47)	---	---	_____
(48-51)	---	---	_____

B. ADULTS: List all places you and your spouse have worked in the past 20 years -

(52) — Do you (have you) work(ed) in lead-zinc related industry?
1 = Yes 2 = No 9 = Unknown

LEAD-ZINC RELATED OCCUPATIONAL CODES

- | | | | |
|---|---------------------------|----|---------------------|
| 1 | Lead smelter worker | 7 | Paint-pigment, zinc |
| 2 | Foundry worker | | copper worker |
| 3 | Oil refinder worker | 8 | Plumber |
| 4 | Painter | 9 | Glass worker |
| 5 | Battery Mfg. plant worker | 10 | Other lead-related |
| 6 | Chemical plant worker | | industry worker |

HUSBAND

	WHEN WORKED			JOB	JOB	
	FROM	TO	COMPANY	DESCRIPTION	CODE	CHEMICALS
	(2)	(2)			(2)	(2)
(53- 60)	_____	_____	_____	_____	_____	_____
(61- 68)	_____	_____	_____	_____	_____	_____
(69- 76)	_____	_____	_____	_____	_____	_____
(77- 84)	_____	_____	_____	_____	_____	_____
(85- 92)	_____	_____	_____	_____	_____	_____
(93-100)	_____	_____	_____	_____	_____	_____

WIFE

	WHEN WORKED			JOB	JOB	
	FROM	TO	COMPANY	DESCRIPTION	CODE	CHEMICALS
	(2)	(2)			(2)	(2)
(101-108)	_____	_____	_____	_____	_____	_____
(109-116)	_____	_____	_____	_____	_____	_____
(117-124)	_____	_____	_____	_____	_____	_____
(125-132)	_____	_____	_____	_____	_____	_____
(133-140)	_____	_____	_____	_____	_____	_____
(141-148)	_____	_____	_____	_____	_____	_____

8. OUTDOOR ACTIVITIES:

1 = Yes 2 = No 9 = Unknown or not stated

(Note to interviewer: Express each as number of times per year (average) even if activity is done only in some seasons.)

(149)___ A. Do you engage in sports that bring you in contact with soil
(e.g., baseball, football, jogging, etc.)?
(150-151)___ If yes, how often per year?

(152)___ B. Do you garden, weed or mow around your home?

(153)___ C. Do you garden, weed or mow someplace else in the neighborhood?

If so, where? _____

9. DIETARY EXPOSURES: 1 = Yes 2 = No 9 = Unknown

(154)___ A. Do you eat vegetables and fruits raised by yourself or in the neighborhood?

(155)___ If yes: Are they root vegetables?

(e.g., carrots, potatoes)

Address where raised if not by (your)self _____

(156)___ Non-root vegetables and fruits?

Address where raised if not by (your)self _____

	Fruit or vegetable	Grown by Self or Neighbor (1)	In season during which months	Average # times eaten/week in season (2)
(157-159)	_____	_____	_____	_____
(160-162)	_____	_____	_____	_____
(163-165)	_____	_____	_____	_____
(166-168)	_____	_____	_____	_____
(169-171)	_____	_____	_____	_____
(172-174)	_____	_____	_____	_____

(175)___ B. Do you eat wild game or fish caught locally?

If yes:(Code: 1 = Mammal 2 = Bird 3 = Fish

	Animal or Fish (1)	Location where caught	Average # times per Year Eaten (2)
(176-178)	_____	_____	_____
(179-181)	_____	_____	_____
(182-184)	_____	_____	_____
(185-187)	_____	_____	_____

- (188) C. Do you eat meat or eggs from farm animals raised in the East Helena area?

If yes:

(1 = beef 2 = pork 3 = poultry 4 = eggs 5 = other)

	Types of meat (or eggs)	Location/ address of farm	Average # times/year
	(1)		(3)
(189-192)	_____	_____	_____
(193-196)	_____	_____	_____
(197-200)	_____	_____	_____
(201-204)	_____	_____	_____
(205-208)	_____	_____	_____

- (209) D. Do you drink milk from cows on farms in the East Helena area?

Address of farm _____

- (210) E. Do you drink water from a private well or spring?

If yes:

	Location of well/spring (2)	No. yrs Used (2)	When discontinued (Year) (2)
(211-216)	_____	_____	_____
(217-222)	_____	_____	_____
(223-228)	_____	_____	_____

- (229) 10. Have you ever sprayed oil around your property to keep down dust?

1 = Yes 2 = No 9 = Unknown

- (230-231) If yes, where did you get the oil?
(Make list) _____

- (232) 11A. Did you ever smoke or use tobacco products?

1 = Yes 2 = No 9 = Unknown

If 'no', go to question 12.

- (233) B. Do you now smoke or use tobacco products?

1 = Yes 2 = No 9 = Unknown

- (234-235) If no, how long ago did you quit? (Years)

- (236-237) C. At what age did you start smoking? (Years)

- (238-239) D. On the average, how many cigarettes do/did you smoke a day?

(240) E. Do/did you usually inhale the smoke when you smoke cigarettes?

1 = Yes 2 = No 9 = Unknown

F. What brand of cigarettes do/did you generally smoke?

12. MEDICAL HISTORY

(241) Have you been treated or diagnosed by a doctor in the past 2 years for any chronic or serious illness?
1 = Yes 2 = No 9 = Unknown

99 = Unknown year

Yr diagnosed	Hospital or physician & address
--------------	---------------------------------

(242-243)	_____	_____
(244-245)	_____	_____
(246-247)	_____	_____
(248-249)	_____	_____
(250-251)	_____	_____
(252-253)	_____	_____
(254-255)	_____	_____

Comments: _____

APPENDIX 7

VENAPUNCTURE

1. Make sure consent form is signed.
2. Educate patient according to their level of comprehension with parent present.
3. Assure patient of minimal discomfort.
4. Inspect patient's arms and hands for best venapuncture site.
5. Determine best method of venapuncture for the patient (butterfly 18 g or conventional needle 21 g assembly).
6. Clean venapuncture site using B-D alcohol preps until alcohol prep shows clean. Let air dry or dry with clean gauze.
7. Be sure patient is properly restrained by parent.
8. Apply tourniquet.
9. Palpate for vein.
10. Insert needle assembly.
11. Draw 2 B-D vacutainer evacuated tubes with EDTA preservative. Mix well; invert 3-5 times.
12. Loosen tourniquet before last tube is full or before withdrawing needle.
13. Withdraw needle.
14. Apply pressure to venapuncture site until bleeding is stopped, then apply band-aid.
15. Write in patient's name and sign labels. Attach labels to tubes.
16. Put tubes in cooler.

Processing Equipment:

1. Consent form
2. Butterfly 18 ga. or 21 ga. by 3/4", 12" tubing infusion set, vacutainer multiple sample Luer-Adapter, Becton Dickinson vacutainer with EDTA preservative, and vacutainer holder.

3. Becton Dickinson (B-D) alcohol swab
4. Tourniquet
5. Cooler with "Blue Ice" packs to keep sample cool
6. Trained and qualified person to obtain blood samples (i.e., medical technician, nurse, etc.)

APPENDIX 8

Hair Collection (Children Only)

1. Instructions for Collecting Hair Samples

- a. Use a new comb and pair of scissors for each subject.
- b. Use disposable, powder-free plastic gloves to handle the hair specimen.
- c. Collect the hair samples:
 - 1) Collect the hair samples from the nape area.
 - 2) With a clean nylon comb, partition the hair between the ears as shown in the diagram.
 - 3) Fasten the hair above the ears out of the way with aluminum clips.
 - 4) At 8-10 sites on the nape area, gather 15-20 strands of hair. Hold the end of the hair and cut the hair as close to the scalp as possible with stainless steel surgical scissors. A minimum of 400 mg is needed for analysis.
 - 5) From each cutting of hair from the scalp, cut off the two inches of hair which were closest to the scalp and store in a Ziploc plastic bag.
 - 6) Discard the remaining length of hair.
 - 7) Seal the Ziploc bag.
 - 8) Affix the appropriate examinee ID number to the Ziploc bag.
- d. Hair samples may be shipped with the other specimens to CDC. Place the hair samples on top of the shipper so that the hair does not get wet.
- e. Disinfect the scissors, clips and combs.

2. Equipment:

- a. Clean nylon combs.
- b. Stainless steel scissors.
- c. Disposable, powder-free plastic gloves.
- d. Aluminum Clips.
- e. Ziploc bags.
- f. Becton Dickinson alcohol swabs.

(1 - 7) I. D. No. _____/_____

EAST HELENA, MONTANA
HAIR COLLECTION QUESTIONNAIRE
AGES 1 THROUGH 5

(8 - 9) 1.
Child No. _____

(10 - 11) 2.
Age _____

(12) 3.
Sex _____

(1 = Male 2 = Female)

(13 - 18) 4.
Date of Collection: Month _____

Day _____
Year _____

(19 - 22) 5.
Sample No. _____

(23) 6.
When was the last time (Child's name)'s hair was washed?
_____ 1 = Today or yesterday
2 = Two through six days ago
3 = Seven days ago or longer

(24) 7.
The last time his/her hair was washed, was it washed at _____
1 = Home
2 = Elsewhere?

(Specify) _____

(25 - 26) 8.
The last time his/her hair was washed, what brand of shampoo was used?
_____ Specify brand _____
99 = Don't know

(27) 9a.
Is this the regular brand of shampoo you use?
_____ 1 = Yes 2 = No

b. If no, what is? _____

(28) 10a.
When washing his/her hair, do you ever use a conditioner or cream
rinse?
_____ (1 = Yes 2 = No)

If no, skip to Question 12a.

(29 - 30) b. If yes, which brand? _____
_____ 99 = Don't know

I. D. # . _____ / _____

(31) 11. How often do you use a conditioner or creme rinse?
_____ (1 = Occasionally 2 = Almost always)

(32) 12a. When washing his/her hair, do you ever use a
dandruff shampoo?

(1 = Yes 2 = No)

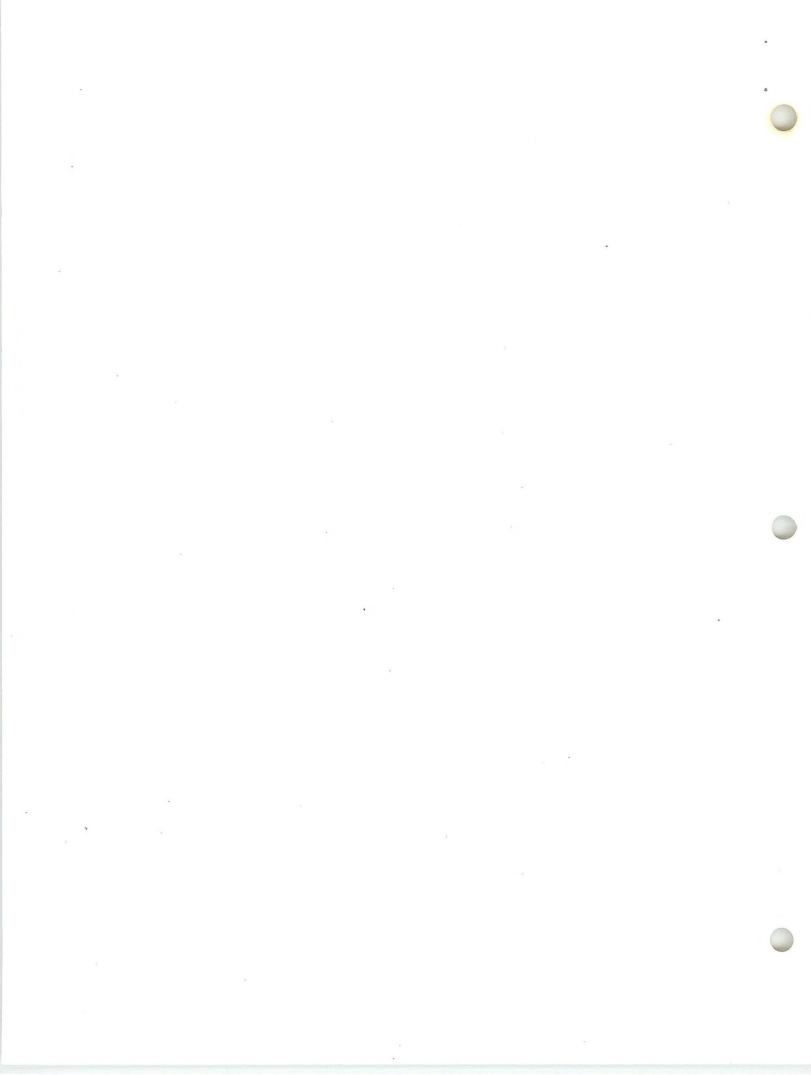
If no, skip to Question 14a.

(33 - 34) b. If yes, which brand? _____
_____ 99 = Don't know

(35) 13. How often do you use a dandruff shampoo?
_____ (1 = Occasionally 2 = Almost always)

(36) 14. Do you ever use any special treatment on his/her
hair, such as hair coloring?
_____ (1 = Yes 2 = No)

If yes, specify _____



APPENDIX 9

Urine Collection (Children and Adults)

1. Instructions for Obtaining Urine Samples

- a. Instruct adults and children (or parents of child) to wash hands with soap and water.
- b. Instruct adults and children (or parents of child) to use a moist towelette (Diaparene) to wash genitalia prior to voiding:
 - 1) Use the towelette once, wiping the labia from front to back or the urethral opening on the penis,
 - 2) Fold the towelette, and
 - 3) Repeat the wiping as in step 1).
- c. Instruct the participants how to collect a clean-catch urine sample:
 - 1) After wiping the genitalia, begin to urinate into the toilet (the first small amount of urine may contain small particles from the opening of the urethra, and we wish to discard this),
 - 2) Collect the rest of the urine in the 132 ml (small cup) specimen container, and
 - 3) Leave the specimen in the bathroom.
- d. Divide the urine specimen into the appropriate tubes as follows:
 - 1) Work over the bathroom sink.
 - 2) Wear the powder-free lab gloves provided in the team kit.
 - 3) For adult samples:
 - a) Place approximately 4 ml into the 6 ml white vial. This is the plain urine sample (no preservatives are in this vial).
 - b) Tap the top of the 50 ml blue-top tube before opening. Once opened, pour up to 25 ml of urine into the tube. The black line on the side of the tube marks the 25 ml level. Cap, label, and invert the tube gently 4-5 times to mix the urine with the small amount (250 ul) of nitric acid present in this tube.
 - c) Tap the top of the 30 ml clear plastic tube before opening. Pour up to 25 ml of urine into the tube. A black line marks the maximum volume for filling on this tube also (i.e., 25 ml). Cap, label as the carbonate sample, and invert the tube 8-10 times until the carbonate is dissolved. (As little as 5 ml of urine is an acceptable volume for this sample.)

- 4) For child samples: collect plain and acid tubes (steps a and b only).
- e. Pour any unused urine into the toilet, rinse the specimen cup, and dispose of the cup in the team's garbage bag.
- f. Collection of field blanks for the acid tubes: For every 25 participants, 3 acid tube field blanks will be collected. If you are to collect such a blank, a second sheet of labels will be in the subject's file and the Urine (Acid) label will be circled. Collection of this blank will be done as follows: At the time of dividing the urine specimen into the appropriate tubes, open an extra blue-topped tube and fill it with deionized water to a level less than the black line mark. Cap and invert as if it were a normal urine sample. Label and store with the other urine samples.
- g. Collection of duplicate samples:
Every fifth participant will have two sets of urine tubes collected. To facilitate this, the following table lists the minimum amounts of urine to be collected:

<u>Tube Type</u>	<u>Best Volume to Use (ml)</u>	<u>Minimum Volume Necessary (ml)</u>
White vial (plain urine)	4	2
Blue-topped tube (acid urine)	25	5
Clear plastic tube (carbonate urine, ADULTS ONLY)	25	5

Thus, for a child participate for whom duplicate samples are needed, a minimum of 14-15 ml must be collected to divide among 4 tubes.

- h. Place the tubes and vials in the shipping containers in the team's styrofoam cooler. Use refrigerator packs in the cooler. Keep all tubes and vials upright. Do not let the tubes touch the ice packs directly.
- i. After the samples have been returned to the operating center and logged in, they must be stored in a freezer at -20 degrees centigrade.

APPENDIX 10

Handwash Sampling

1. Instructions for Collecting Handwash Samples

- a. Be sure consent form is signed.
- b. Educate parent as to the strength of the acid. Assure parent that weak acid will not harm child provided hand is rinsed well in tap water immediately after dipping.
- c. Educate child according to their level of comprehension.
- d. Inspect child's hands for open wounds or cuts, and if present, do not dip that hand.
- e. Dip one hand into wide mouth plastic container containing approximately 500 ml of 0.1 M nitric acid with fingers slightly moving for 20 seconds (use stopwatch). Remove hand from container and immediately rinse well with running tap water. Dry hand. Repeat above procedure on other hand.
- f. Recap sample as quickly as possible to avoid any contamination.
- g. Label appropriately - note any nail polish and its color on the sample sheets.
- h. Handwash Blanks.
 - 1) Take one extra handwash jar with you when you do the child's hand dip.
 - 2) Either before or after child's hands are dipped, open blank hand dip jar for 40 seconds. Recap jar and label accordingly.

2. Processing Equipment

- a. Wide mouthed quart polyethylene mason jars (Bel Arts)
- b. 500 ml of 0.1 normal nitric acid
- c. Stopwatch
- d. Paper towels



APPENDIX 11

Soil Collection

1. Soil Sampling Procedure

a. Instructions for Selecting Soil Sample Locations

1) Front of House

The first soil sample will be taken in the front yard. Estimate the approximate center of the yard when facing the house, move one and one-half meters (1.5) from the curb and draw a circle one meter in diameter from that point. Samples will be taken at four equally spaced locations in the circle, one at the northern most point in the circle, one at the southern most point, one at the eastern most point and one at the western most point.

If it is not possible to take a sample at the center of the yard because of a walkway, driveway, etc., while facing the home, move one meter to the right of the obstruction and proceed. In the event it is not possible to draw a circle, measure and collect four subsamples from a rectangular configuration one-half meter (.5) by two (2) meters. Soil collected at each corner of the rectangle will be composited identical to those collected from the sampling circle. All samples are obtained by compositing the four subsamples from the circle or from the four corners of the rectangle.

If there is no well defined curb, as is the case in most of the East Helena area or of an unpaved street, the edge of the pavement or gravel will be used as the curb. If there is some obstruction one meter from the curb such as a sidewalk or drainage ditch, move toward the house and to the right until the circle or rectangle can be established.

2) Rear of House

The second soil sample will be taken in the rear of the house in the back yard. If there is no back yard, then the soil sample should be obtained in whatever open space is available, but at least six meters from painted surfaces such as walls of house, shed, or painted fence.

Samples from the front and rear of the home or residence will be composited for sample handling and analyses.

3) Side of House

Estimate the approximate center of the house, front to rear on one side and draw the one-meter circle 1/2 meter from the foundation of the house. This should yield one subsample immediately adjacent to the foundation, two subsamples half a meter from the foundation and the fourth subsample one full meter from the foundation.

This "side of house" procedure will be used to collect one sample from significant structures which are adjacent to the residence such as garages, shops, etc., and one sample (using the one meter circle method) from a major bare soil play area, if lead in paint is evident.

The environmental technician will be instructed to sketch the house, grounds, and soil sampling sites on the Environmental Survey form in order that the soil sampling sites may be identified.

4) Other Public Areas

In multiple dwelling housing units it will not be possible to obtain three composite soil samples for each eligible child. In these instances, the areas in front and rear of the apartments should be sampled, composited, and recorded. One "side of building" sample will be collected. Playgrounds and other areas held in common by the residents and available to the children must be sampled, using methods described above.

5) Play Areas

If a playground or vacant lot adjacent to the home is a favorite play area, it will also be sampled. The sampling will be based on whether a child plays more than 50 percent of the time in the adjacent play area, as determined by the parents. On these adjacent playgrounds, lots, or other areas not related to a dwelling, the sampling scheme to be followed is a modification of that previously discussed. Draw an imaginary line to the opposite diagonal corner of the playground or lot. Along this line every ten (10) meters take samples in the manner prescribed. Follow the same procedure for the other two corners, making sure to take a sample in the center where the lines intersect.

It is necessary to clearly record the playground sites since addresses will not identify them adequately. The distinction between bare earth samples and those in grass or sod covered areas must be made in order to determine the relative contribution each type might make to blood lead levels. Any bare areas will be sampled separately.

If children play in a neighbor's yard, soil samples will be collected in a manner as above after permission is obtained.

b. Instructions for Collecting Soil Samples

- 1) Determine the appropriate area for sampling as described above (Sample Locations).
- 2) Place 0.7 m² wooden template on the ground and orient arrow (on template) to point north with the aid of a compass.
- 3) Insert acid washed acetate liner in JMC "O" Contamination Tube, and affix "O" Contamination Tube to the JMC Backsaver N-2 Handle.
- 4) Sink "O" Contamination Tube vertically into the soil at one corner of the 0.7 m² wood template. Withdraw in a vertical manner after achieving desired core depth (approximately 2 inches). Proceed to the next sample corner (until all corners have been sampled) and repeat this step. Thus, the sample cores will be contained within one or two acetate tubes, depending on depth of individual cores.
- 5) Remove acetate liner containing the sample from the "O" Contamination Tube. Put acid washed caps on both ends of the acetate liners are required to obtain the sample and/or duplicate, they should be taped together with proper identification labels on each acetate tube.
- 6) Store in an upright position to preserve soil core stratification and to minimize mixing.
- 7) Clean "O" Contamination Tube by placing in a tap water bath and using a large test tube brush to scrub the inside surfaces free of organic soil and clay. Visually inspect inner surfaces of "O" Contamination Tube (and nozzle, particularly) to ensure soil has been removed. If soil remains, repeat this step.
- 8) Rinse with distilled de-ionized water and then with reagent grade acetone to aid in drying the inside of the "O" Contamination Tube.
- 9) Use clean paper towels to wipe the exterior surface of the "O" Contamination Tube clean and dry. Periodically check the nozzle by wiping with a B-D alcohol swab to insure cleanliness at that critical interface.
- 10) Place dried "O" Contamination Tube in heavy duty Ziploc bag and proceed to the next sample area.
- 11) Prior to in-field sampling, computer-aided random number generation will be used to ascribe duplicate and blank control procedures for 18.5% of the sample area.

- 12) Duplicates: After obtaining cores as per the procedural steps 1-11 above, rotate the 0.7 m^2 template 90° and extract duplicate cores (approximately 0 to 3.5 inches in depth).
- 13) Blanks: After the in-field cleaning procedures rinse the "O" Contamination Tube with distilled-deionized water. Collect the DI rinse water in an acid-washed polyethylene container with a water-tight screw cap. Label the containers appropriately and store in an upright manner.
- 14) For sandy soils, and sand boxes in particular, use the acetate liner and place a clean gloved hand at the bottom of the acetate sleeve such that the contents remain in the liner upon extraction. Composite these samples in whirl-pak bags, label and store in a separate box.

2. Soil Sampling Equipment

- a. JMC Backsaver N-2 Handle.
- b. "O" Contamination Tube.
- c. Acetate liners and caps.
- d. Wood template measuring 0.7 m^2 .
- e. Polyethylene gloves.
- f. Tap water bath.
- g. Large test tube brush.
- h. Distilled - deionized water.
- i. Reagent grade acetone (Baker analyzed).
- j. Becton Dickinson alcohol swabs.

APPENDIX 12

Dust Collection

1. Instructions for Collecting Household Vacuum Bag Samples

- a. Depending on vacuum type, reach in or rip open vacuum bag to obtain sample.
- b. Using plastic gloved hand, obtain a large handful of vacuum bag contents.
- c. Transfer to Ziploc plastic bag.
- d. Label appropriately.

2. Instructions for Collecting Special Vacuum Cleaner Samples

a. Description of Sampling Apparatus.

A high-volume airborne particulate sampling device (Sierra or General Metals) with an intake flow of about 55 cubic feet per minute has been modified so a plexiglas hood can be attached to the horizontally positioned stainless steel filter holder. Two micron pore size Zeflour 8 by 10 inch teflon filters will be used on the device to insure collection of the very fine inhalable particles (down to about 0.01 micron size) which are not retained by a typical household vacuum cleaner. Smooth plastic dust attachments along with clear plastic (tygon) 1-inch, thick-walled, 8-foot tubing lengths will transfer floor dusts to the hood of deposit onto the teflon filter.

b. Sampling Procedure

- 1) Be sure hood-hose-wand attachments are clean. Check to see that all parts have the same equipment number.
- 2) Be sure that sample transport box is clean and filled with prenumbered and bagged teflon 8 by 10 inch filters with back-up glass fiber filters.
- 3) Be sure sufficient cleaning items are available: 5 gallons of deionized-distilled water, brushes, paper towels, ethanol, clean bags, gloves.
- 4) Allow 20-25 minutes per home for sampling.
- 5) Upon entering the home, identify a high-traffic carpeted area between the living area and the kitchen which is away from an outside entrance if possible.
- 6) Wipe the wooden 1-square meter template with an ethanol soaked towel and dry, then position it on the floor with enough room for the worker to gain access to two sides of the template.
- 7) Clean the stainless steel filter holder with an ethanol towel, dry and load the vacuum device with a teflon/glass-backed filter pair, retaining the labeled filter-holder bag for the about-to-be loaded filter. Log the ID numbers, residence address, etc., on the data sheet.

- 8) Position the vacuum device adjacent to the template: kneel on the plexiglas template so it remains stationary; turn the vacuum blower motor on; simultaneously begin dust collection when the stopwatch is started; vacuum vigorously for 30 seconds; stop the time without resetting it; reposition yourself on the template perpendicular to the original position and repeat the collection of dust for 30 seconds.
- 9) Remove the sample hood. With gloved hands gently raise the edges of the filter paper to prevent dust from falling off the filter and carefully reinsert the loaded filter into the original plastic bag before sliding it horizontally into a slot in the transport box.
- 10) Disassemble the vacuum apparatus placing the soiled hood, hose, adaptors, etc., into the original bag for return to the laboratory for cleaning. Outside the residence, attach a hose on the vacuum blower motor exhaust to blow the filter holder device clean, then wipe the stainless steel filter holder with an ethanol towel and dry.

APPENDIX 13

XRF Analyses of Painted Surfaces

1. Instructions for Conducting Sampling

- a. Explain basic purpose and function of the XRF to the property owner. Answer any questions concerning the XRF and obtain verbal consent for use of XRF.
- b. Ask parents if there are any painted surfaces that the children put their mouths on that should be checked by XRF. Label and fill in all data necessary on XRF log sheet.
- c. Check XRF for accuracy using the lead standard supplied with each instrument. Run standards at each house before and after taking household readings.
 - 1) To run the standard - place lead standard on floor; place XRF on standard; press trigger. Take three readings and record the middle value, being careful to note that the readings are within specified standard deviations stated on the lead standard. Record results.
 - 2) If standards are not within recommended standard deviations, calibrate according to manufacturer's recommendations.
- d. Readings should be taken in all areas where the children spend a lot of time and where the children can reach - approximately 3 feet from the floor. Take three readings at each station and record the middle value.
 - 1) Take readings on exterior of house, 1 foot from the right doorframe, and 3 feet from the surface on which you are standing (ground or porch floor). Take readings on back, front, and side doors, according to amount of use. If surface is unpainted brick, or other surface without a coating, take a reading on the door.
 - 2) Take readings on one site in each room on a painted wall surface. Readings should be taken throughout the house: in the kitchen, all bedrooms, bathrooms, hallways, dining room and living room. If walls are not painted, a reading should be taken on the trim.
 - 3) Other places readings should be taken are on painted children's furniture and toys, fences, sandboxes, exterior buildings, and fences.
- e. Note chipping and peeling on trim and/or wall at each station site, and record results.
- f. Take reading on lead standard and record value.
- g. Turn off XRF.

2. Processing Equipment:

- a. Portable XRF with lead standard for calibration and precision purposes. Princeton Gamma Tech portable XRFs (Model XKJ) will be used, with U. S. Department of Housing and Urban Development lead standards.
- b. Battery packs for operation of the XRF in the field.

EAST HELENA, MONTANA
XRF READINGS

(1-7) I.D. NUMBER _____ / _____
(Block #) (House #)

(8) Area _____ Logged _____

PAINT

INSTRUMENT NUMBER	XRF STANDARD	XRF READING AT START	XRF READING AT END
(9 - 11)	(12 - 15)	(16 - 19)	(20 - 23)

(Circle correct numbers)

AREA	WALL CHIPPING OR PEELING			TRIM CHIPPING OR PEELING			XRF SITE		XRF READING
	Yes	No	NA	Yes	No	NA	1 = Wall	2 = Trim	
(24 - 31) 1.Living Room	1	2	8	1	2	8	1	2	___.
(32 - 39) 2.Bedroom 1	1	2	8	1	2	8	1	2	___.
(40 - 47) 3.Bedroom 2	1	2	8	1	2	8	1	1	___.
(48 - 55) 4.Bedroom 3	1	2	8	1	2	8	1	1	___.
(56 - 63) 5.Bedroom 4	1	2	8	1	2	8	1	2	___.
(64 - 71) 6.Bathroom	1	2	8	1	2	8	1	2	___.
(72 - 79) 7.Kitchen	1	2	8	1	2	8	1	1	___.
(80 - 87) 8.Dining Room	1	2	8	1	2	8	1	1	___.
(88 - 95) 9.Hallway	1	2	8	1	2	8	1	2	___.
(96 - 104) 10.Exterior	1	2	8	1	2	8	1	2	___.
(105 - 113) 11.Other (Specify)	1	2	8	1	2	8	1	1	___.
(114 - 122) 12._____	1	2	8	1	2	8	1	1	___.
(123 - 131) 13._____	1	2	8	1	2	8	1	2	___.
(132 - 140) 14._____	1	2	8	1	2	8	1	2	___.

DATE: _____ INSPECTOR: _____

Floor Wipe Samples

1. Instructions for Collecting Floor Wipe Samples

- a. Determine sample site. Take dust sample only on a smooth surface (no rugs or carpeting) on floor below the site used to take the kitchen XRF reading.
- b. Remove template from plastic bag. Handle template by the outside so as not to contaminate the inside edges.
- c. Lay template over sample site.
- d. Open two alcohol swabs as follows:
 - 1) Before tearing open alcohol swab, press the package from top to bottom forcing the swab toward the bottom away from the dotted line.
 - 2) Tear along the dotted line and discard the smaller portion of the package.
 - 3) Invert package and squeeze out all the alcohol from the package.
 - 4) Carefully tear the two sides of the package off, being careful not to touch or tear the swab.
 - 5) Lay completely opened package on a convenient chair or table.
 - 6) Open a second alcohol swab in the same manner.
- e. Fold swab into a triangle. Grasp one corner of the swab, being careful to touch as little of the swab as possible, then grasp the opposite corner until you have the tips of both corners between your thumb and index finger.
- f. Begin floor wipe. Place the triangular pad in inside upper right corner and move to the left, moving in one direction only. When you come to the left side of the template, lift the pad, go back to the right side, place pad directly underneath first pad path and repeat procedure until the entire area within the template has been covered.
- g. Place the pad with the dust covered side up on the inside of the used package.
- h. Using second alcohol swab as described above, make a series of pad wipes from top to bottom until entire surface has been covered.
- i. Place second dust covered pad on top of the first with the soiled surfaces facing each other.
- j. Close the top of the package and roll toward the closed side.
- k. Place sample in Ziplock bag.
- l. Label appropriately.

2. Collection Equipment:

- a. Plexiglass template framing a 400 cm² area.
- b. Becton-Dickinson low metal alcohol swabs.
- c. Ziploc bags.
- d. Gauze.
- e. Travenol vinyl gloves.



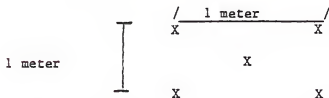
Garden Vegetable Collection

1. Garden Selection

Select one garden plot (if available) per city block for sampling. Select a well-ventilated garden plot in the southern portion of each block. In residential areas without city blocks, such as in certain subdivisions and on farms, select gardens which are representative of the area.

2. Instruction for Collecting Garden Samples

- a. Wear a pair of previously unused plastic gloves.
- b. Collect approximately 400 grams each of 3 types of vegetables:
 - 1) Type 1 - Leafy vegetable, such as lettuce and cabbage leaves
 - 2) Type 2 - Above ground vegetables, such as peas and beans
 - 3) Type 3 - Underground vegetables, such as carrots and radishes
- c. Collect samples of leafy garden vegetables by clipping the plant above any visible splash line or at least four cm from the ground. Take only the edible portion of the plant.
- d. Wear new plastic gloves for each vegetable type. Grasp the plant with one hand and clip it with the other hand. Collect samples from the middle and about 0.5 meters from each end of a patch or row.
- e. Place samples in brown paper kraft bags to prevent sweating, date and label with preprinted labels for delivery to the operations control center.
- f. For each garden sampled, collect five 10 cm deep soil samples for compositing. Sample according to the diagram shown below:



- g. Place the composite sample in a Ziploc plastic bag, date, label, and deliver to the operations center.



APPENDIX 16

EAST HELENA, MONTANA
SOIL AND VEGETATION SAMPLE

(1 - 7) I.D. # / _ _ _ / _ _ _ _ _

PLACE LABEL
HERE

SOIL AND VEGETATION SAMPLE

(Circle one)

		Yes	No	Not Applicable	Unknown
(8)	1. Front Yard	1	2	8	9
(9)	2. Back Yard	1	2	8	9
(10)	3. Side of House	1	2	8	9
(11)	4. Play area	1	2	8	9
(12)	5. Other location	1	2	8	9
	If other, specify				
(13)	6. Adjacent play area (Vacant lot, etc.)	1	2	8	9
(14)	7. Garden soil composite	1	2	8	9
(15)	8. Adjacent structure soil	1	2	8	9
(16)	9. Kitchen dust sample	1	2	8	9
(17)	10. Vacuum carpet sample	1	2	8	9
(18)	11. Household vacuum bag dust	1	2	8	9
(19)	12. House cleaning:	1 = Poor		3 = Average	
		2 = Fair		4 = Excellent	

I.D. # _____

13. Percentage of grass cover:		0%-25%	26%-50%	51%-75%	76%-100%
(20)	Front yard	1	2	3	4
(21)	Back yard	1	2	3	4
(22)	Play area	1	2	3	4

Date: _____ Inspector: _____

Sketch the placement of the house on the property. Identify as accurately as possible the location of each soil sample.

Letter to parents of children ages 1 through 5 living in Area III (Helena)

Address

Dear Mr. and Mrs. :

You may be aware that the Montana Department of Health and Environmental Sciences is conducting a study to investigate the possible lead exposure of pre-school children living in the East Helena area.

A study of this nature requires a control group which means a separate, nonexposed population to compare with those having probable lead exposure. A section of Helena has been chosen as the control area. This letter assumes that you live in this area.

A previous house to house survey indicates that you have one or more children in the age range of 12 months through 5 years.

The study would consist of an interview, obtaining samples of blood, urine and hand dust, measurement of lead in paint, and collection of dust within the house and soil and from the yard.

It is not expected that significant amounts of lead will be found in your child/children or in your house and yard. In any event, you will be given the results of the tests.

If you have any questions, please call the Air Quality Bureau at 449-3454.

You will soon be receiving a telephone call requesting an appointment to visit your home.

Your cooperation is appreciated.

Sincerely,

John J. Drynan, M.D., Director
DHES



Laboratory Methods - - Environmental Samples

1. Instructions for Soil Samplesa. Sample Preparation

- 1) Place clean white paper on work table.
- 2) Orient acetate liner lengthwise on clean paper. Remove both caps from liner.
- 3) Use PVC plunger (PVC pipe with silicone stopper) to push sample out of acetate sleeve. Apply just enough pressure with PVC plunger to extract sample. There will be variation between samples as to the force necessary for core removal. Make sure to grip acetate sleeve firmly during this step.
- 4) Dissect sod layer, if present, from core. Remove the sod layer at that zone where "crumbly soil" meets "compacted soil." Thus, remove the portion from the top of the compacted soil zone up through the surface vegetation (1 to .25 inches), as follows:
 - a) Determine the depth of the sod layer by examining soil structure.
 - b) Use clean heavy duty serrated plastic knives for the separation process.
 - c) Use plastic knives for one separation and then compile them in a box such that they may be washed.
 - (i) Wash plastic knives with soap and tap water, then give a final rinse with distilled-deionized water.
 - (ii) Let air dry and re-use.
- 5) After removing sod for all cores of a given sample (four), composite the sod tufts in a Ziploc bag and label appropriately. Store in a box. If there is no sod layer present, one-inch segments may be measured from the core without prior surface soil removal.
- 6) Place a plastic ruler along the length of the core. Remove one inch of soil beginning at the compacted soil zone just below the sod layer and composite these one-inch segments in a labeled paper Kraft bag for a given sample (not duplicates).
- 7) Discard the rest of the soil.
- 8) Break up composited one-inch sample segments in labeled paper Kraft bags by squeezing segments while in the bag (using paper bag as a buffer between hands).
- 9) Place Kraft bags containing composite sample in oven at 80°C for 24 hours. This will yield data on a dry-weight basis as processing to the analysis stage proceeds.
- 10) Duplicates should be treated just like samples, with the following exception. After removal of the sod layer (if apparent), instead of measuring off a one-inch segment, measure off a three-inch segment. Composite these

- three-inch units in a labeled Kraft bag (with additional "duplicate" identification). Break up the segments as per step #8, and place in oven to dry as described in step #9.
- 11) Pass the 0-1 inch samples and 0-3 inch duplicates through a 10, 2 millimeter (Tyler equivalent of a 9 mesh) sieve by mechanical dry shaking on a Gilson Sieve Tester for 5 minutes.
 - 12) Transfer sieved soil from the sieve catch pan to Whirl-pak polypropylene bags and affix proper identification labels.
 - 13) Clean the Gilson Sieve Tester as follows:
 - a) Run the putty knife on both surfaces of the two sieves. Blow out with breathable compressed air.
 - b) Brush with plastic nylon dish brush on both sides of the two sieves. Blow out with breathable compressed air.
 - c) Use a moist ethanol paper towel to gently rub the bottom of the two sieves. Blow out with breathable compressed air.
 - d) For the catch pan, first blow it out with the compressed breathable air, then wipe clean with a moist ethanol paper towel. Use the compressed air once again to dry the pan.
 - e) Wipe top of Gilson shaker with a dry paper towel, and a quick blast of breathable compressed air.
- b. Instructions for Using SPEX Shatterbox Swing Mill Procedure
- 1) Dry samples prior to grinding by placing the Whirl-pak bag in a drier where the temperature is between 35-40° C with the bag opened to allow moisture to escape. Samples are to be dried a minimum of 6 hours.
 - 2) Weigh 100 milligrams \pm 10 mg of Ivory Snow (sodium stearate) into a plastic weighing dish for aid in grinding.
 - 3) When extracting sample material to grind, lightly shake the Ziploc bag such that the sample moves to one corner of the bag. Run the pre-cleaned (ethanol and paper towel) stainless steel spatula down the inside seam to the corner of the bag for a representative sample. Gently shake the spatula while still within the sample bag if too much sample is on the spatula, in order to minimize sample loss.
 - 4) To the grinding aid in the weighing dish, add 5.000 \pm 0.0010 grams of sample. Record the residence identification number (the three digits following the prefix 312-____) both on the side of the plastic vial and on the vial cap. Following the sample ID, indicate original sampling location by the code:
Front = A
Side = B
Play = C
Adjacent play area = D
Adjacent structure = E
Garden = F
Other #1 = G
Other #2 = H

An example: 312-167A represents a front yard sample collected from residence 167.

- 5) Care must be exercised in handling large numbers of samples to prevent mislabeling or sampling the same Whirl-pack bag twice. Establishing sample handling routines and work bench holding locations will prevent such problems.
- 6) Pour the 5 gram sample plus 0.1 gram grinding aid into a previously cleaned SPEX shatterbox tungsten-chromium swing-mill grinding container. Place sample between the center puck and the four inch ring to prevent spilling the sample. Once most of the sample is placed in the grinding container, invert the plastic weighing dish over the puck and tap the bottom to insure the total sample is transferred to the grinder. Make sure the rubber O-ring is properly seated before placing the lid on the grinding container. Carefully place the grinding container into the shatterbox (onto the rubber mat) and clamp the 12 inch pivoting arm over the container such that the clamp centers on the grinding container lid. Clockwise tighten the clamp knob until it can no longer be tightened with two hands and lift the locking device to prevent the knob from working loose during the grinding process. Close the soundproofing shatterbox lid, turn the toggle switch to on, rotate the timer to 5 minutes and press the white button on the timer knob. Grinding will proceed and stop automatically.
- 7) Once the grinder stops, press the locking device to loosen the tightening knob. Lift the unopened grinding container from the shatterbox with two hands and place it on a clean sheet of 8 1/2 x 11 inch paper in the hood. Wear latex gloves and a face mask during this process.
- 8) Slide and carefully lift the lid off the container and hold it vertically on a second clean sheet of 8 1/2 x 11 inch paper. In this position gently brush the fine dust on the lid down with single strokes, using a 1" nylon paint brush, onto the center of the sheet of paper. During this operation, the hood vent fan must be turned off to prevent loss of the sample. Gently set the heavy grinding container lid aside. Brush the ring and puck with increasingly smaller concentric circles in order to remove sample material from the top of the puck and 4" ring. Additionally, brush the exposed sides of both the ring and puck while in the grinding bowl. Brushing in this manner will result in concentrating the sample in the bowl, and facilitate sample recovery and the cleaning process. With two hands, tilt either the puck or the ring from the grinding container so it can be lifted onto the sheet of paper. Gently brush all sides down, collecting the sample on the center of the sheet of paper.

- 9) Fold the middle of one side of the sheet of paper which holds the sample and carefully funnel the sample into a previously cleaned and labeled vial. Cap the vial to secure the sample and check it off from the printout as being completed. Discard the plastic weighing dish and the sheets of paper.
 - 10) Use a vacuum (or breathable compressed air) to move dust from the grinding container, then moisten a paper towel with ethanol and wipe all interior grinder surfaces. Repeat this process until the alcohol towel shows no further soiling. The rubber O-ring should be removed and cleaned without undue stretching so as to extend its lifetime. Re-assemble the set-up for the next sample.
 - 11) If towel wiping the inner grinding parts does not prove sufficient, scrape the surfaces with the stainless steel Chicago Cutlery spatula and repeat towel wipes. If this still does not visually accomplish the cleaning, pour about 5 grams of sand (Sargent-Welch 99.8% pure) into the grinder and set it up to run for 2 minutes. Upon completion clean the device as per 9. above.
- c. Instructions for Using SPEX X-Press (Pelletizer) Procedure
- 1) Assemble press components in preparation for soil samples.
 - 2) Pour ground sample into aluminum sleeve. It may help to rotate the plastic sample container and gently tap it while pouring into the aluminum sleeve.
 - 3) Slowly insert aluminum plunger into aluminum sleeve.
 - 4) Stabilize press components and apply pressure to aluminum plunger while firmly holding down the aluminum sleeve.
 - 5) Firmly grip the aluminum sleeve with one hand and the aluminum plunger with the other hand.
 - 6) Begin to extract the aluminum sleeve holding the aluminum plunger in place. This will free the pellet from the sleeve. The sleeve should be withdrawn about 1/4 of an inch prior to extracting the aluminum plunger in unison with the aluminum sleeve. This can be a continuous motion.
 - 7) After extracting aluminum sleeve and plunger, see that the sample is intact in pellet form.
 - 8) Pour a level tablespoon full of boric acid in the pellet chamber, carefully, so as not to disturb the integrity of the pellet form.
 - 9) Insert machined cylinder press; secure press chamber base and lightly press down on machined cylinder.

- 10) Place press chamber components in press, holding secure the bottom plate, mid-chamber and machined cylinder.
- 11) Screw down securing threads tightly on machined cylinder, holding the press components securely.
- 12) Tighten hydraulic fluid grip (clockwise) and turn press on. Watch the pressure gauge, and when it reaches 15 tons, count for 2 seconds and turn press off.
- 13) Let pressure decrease moderately, then slowly turn hydraulic fluid grip counterclockwise. Observe pressure gauge as you turn the hydraulic grip, as the pressure decrease should not be drastic.
- 14) Raise the securing threads via the wheel on top of the press and remove press chamber components in the same manner as they were placed into the press. That is, holding the bottom plate, mid-chamber and machined cylinder securely.
- 15) Carefully stand press components on machined cylinder and remove the bottom plate (which is now the top). Put PVC tubing where bottom plate was, and place once again into the press. Align the washer with the securing threads and continue to tighten. Hold the mid-chamber of the press components securely until pellet is extracted from the chamber.
- 16) Remove press components carefully, holding the machined cylinder at the bottom so it will not fall out.
- 17) Remove PVC tube and grab pellet with gloved fingers. Be careful not to touch soil surface of pellet. On the "top" of the pellet, boric acid side, write the sample three digit # and alpha coding (as was on plastic vial). Record the date on the pellet as well and then place in foam "nest", soil side facing down.
- 18) Use air hose on all pellet chamber components under the hood, aiming at the exhaust duct.
- 19) Then use ethanol and paper towel (Hi Dri) to wipe components clean of sample dust. The aluminum sleeve, aluminum plunger, mid-chamber and the bottom plate should be given special attention.
- 20) Blow out components and assemble as they are air cleaned. The following order may be used: bottom plate, mid-chamber, aluminum sleeve, aluminum plunger, machined cylinder.

d. Sample Analysis Methodology

Pelleted soils were analyzed by energy dispersive X-ray fluorescence (XRF). This method involves the excitation of characteristic X-rays in the sample by an external source of radiation. Calibrated measurements of the intensities of these X-rays quantify the sample's elemental concentrations. The XRF system used was EG&E ORTEC TEFA III. Calibration consisted of (1) entering standard concentrations with associated uncertainties into computer files; (2) selecting metal filter types to increase background to peak ratios; (3) setting regions of interest for all desired elemental peaks including several regions for interfering elemental

corrections; (4) running the standards to obtain spectra; (5) performing absorption/enhancement corrections and stripping peak overlaps caused by interfering elements; and (6) adjusting the computer for an appropriate report format. Once the XRF system was calibrated, samples were run in batches of 24 by entering identification numbers and initiating the computer-controlled analytical run. The duration of each run was 6 hours.

The first position sample in each batch was a standard used for normalization to the previous calibration. The last sample in each batch was a reference sample used for comparisons. Samples were subjected to 300 second live-time counts to optimize the statistics of the counting process. The area of the pellet surface impacted by the X-ray beam was approximately 1 square centimeter. The small soil particle size (less than 50 micron diameter) within the pellets eliminated the particle size corrections ordinarily needed to determine the concentrations of low atomic number elements. Wet-chemical analyses were performed using (1) a Perkin-Elmer sequential ICP-5500 system for inductively-coupled argon plasma analyses; and (2) a Varian AA6 equipped with a hydride generator for obtaining arsenic values.

e. Equipment

1) Sample Preparation

- a. Plastic serrated knives.
- b. Nasco Whirl-pack bags.
- c. Kraft bags (lunch bag size).
- d. Gilson Sieve Tester, Model SS-15.
- e. No. 10, 2 millimeter mesh sieve (Tyler equivalent to a 9 mesh).
- f. Sieve catch pan.
- g. Clean white paper.
- h. 95% ethanol and paper towels.

2) Grinding

- a. SPEX Shatterbox cat. no. 8510.
- b. SPEX Shatterbox tungsten-chromium grinding dish with ring and puck, cat. no. 8504.
- c. Whirl-pack polypropylene bags.
- d. Latex gloves.
- e. 1" nylon brushes.
- f. 13 dram plastic vials.
- g. Breathable compressed air.
- h. 8 1/2" x 11" paper.
- i. Paper towels.
- j. 95% ethanol.
- k. Sargent-Welch 99.8% pure sand.
- l. Ivory Snow (stearic acid 99%).

3) Pelletizing Equipment

- a. SPEX X-Press cat. no. 36248.
- b. SPEX X-Press machined components.
- c. Machined aluminum sleeve and dowel.
- d. Latex gloves.
- e. Boric acid.

- 12) The catch pan samples are then processed in a similar fashion as soil samples. The SPEX swing mill and SPEX X-Press procedures outlined in the soil subsection should be followed. Grinding time for vacuum samples, however, is 2 minutes, instead of 5 minutes.
 - 13) For the vacuum samples less than 5 grams, it is important to maintain the ratio of 5 grams of sample to 0.10 grams of stearic acid (for matrix similarity in XRF analyses). If sieved vacuum samples are equal to or less than 2.5 grams, they need not be processed further.
 - 14) Clean the sieve network, as per Soil Samples a.14., above.
 - 15) Assemble sieve network and ready for the next sample.
- b. Processing Equipment:
- 1) Gilson sieve tester SS-15.
 - 2) 2.0 millimeter sieve.
 - 3) 850.0 micrometer sieve.
 - 4) Sieving catch pan.
 - 5) Stainless steel spatula.
 - 6) 13 dram plastic snap cap prescription vials.
 - 7) Breathable compressed air.
 - 8) Plastic nylon brush.
 - 9) 2 inch putty knife.
 - 10) 95% ethanol.
 - 11) Hi-Dri paper towels.
 - 12) Ziploc bags.

3. Garden Vegetable Samples

a. Sample Preparation Methodology - Drying

In the laboratory, vegetables were individually removed from kraft bags (with plastic gloves), dipped into a stainless steel bowl containing six liters of cold tap water, stirred lightly while intermittently submerging for 30 seconds each composite sample, then rinsed once in running tap water. This step was intended to be similar to the vegetable cleaning done in the home. Vegetable samples were placed in individual beakers for drying at 80°C for a minimum of 48 hours. Samples were then ground in a Wiley mill to pass a 40-mesh screen and ashed.

b. Sample Preparation - Methodology Digestion

The University of Montana, Missoula, MT, used the following digestion procedure for preparing garden for vegetation rinse samples. The procedure is appropriate for preservation of potentially volatile metals, as adapted from Van Meter (1974), and uses the following instructions:

- 1) Weigh out 0.25 g of sample.
- 2) Place in 2.5 cm by 25 cm acid washed Pyrex test tube.
- 3) Add ten milliliters of intra-analyzed, concentrated nitric acid.
- 4) Seal tube with an oxygen-methane torch.
- 5) Place sealed Pyrex tube in sections of steel pipe, and cap both ends.
- 6) Put capped steel pipe sections into 150°C oven for three hours.



- 7) After cooling, the Pyrex tubes are removed from the steel pipe sections and depressurized by opening a small vent hole with the oxygen-methane torch. This should be done under a hood with all the precautions of working with concentrated acids (i.e., apron, protective glasses, gloves, etc.).
 - 8) Score the Pyrex tube, wipe with trace metal free B-D alcohol swabs and remove the top of the Pyrex tube along scored groove.
 - 9) Transfer solution into 100 ml beakers using a concentrated nitric acid squirt bottle to aid in sample solution recovery from the Pyrex tube.
 - 10) Evaporate sample solutions to dryness on a heat plate (under an acid hood) with gentle heat to remove the concentrated nitric acid.
 - 11) Re-dissolve dried samples with 0.3 Molar intra-analyzed nitric acid to a final volume of 10 ml in volumetric flasks.
 - 12) The 10 ml of final solutions were then transferred to plastic screw-top vials.
- c. Laboratory Analyses of Garden Vegetable Samples
MDHES analyzed the vegetable rinse samples by AA and ICAP to determine the sample concentrations of lead, cadmium, arsenic, zinc, and copper.
- d. Equipment
- 1) Sample Processing
 - a. 2.5 cm by 25 cm acid washed Pyrex test tube
 - b. Intra-analyzed concentrated nitric acid
 - c. Oxygen-methane torch
 - d. Steel pipe sections with threaded caps
 - e. Oven capable of 150°C
 - f. Glass scorer (a zinc plated one was used in this procedure)
 - g. Trace metal free B-D (Becton Dickinson) alcohol swabs
 - h. 100 ml Pyrex beakers
 - i. Hotplate
 - j. Plastic screw top storage vials (10 ml)

Reference:

Van Meter, W. Heavy Metal Concentration in Fish Tissue of the Upper Clark Fork River, Montana University Joint Water Resources Research Center, Bozeman, MT (1974).

4. Summary of Environmental Specimen Analyses

Table A lists the sizes of the samples analyzed for different environmental specimens. The type of sample treatment, the standard reference materials used, and the method of handling the data from these analyses. Table B lists the minimum laboratory detection limits along with ICP/AAS wavelengths for the elements described within this report.

Instrumental X-ray calibrations were difficult because standards were difficult to find which fell within the high range of expected soil concentrations. For this East Helena study, we expected that elemental soil lead concentrations would almost reach 10,000 ppm. National Bureau of Standards soil and bulk reference materials contain only low lead concentrations except for river sediment (at 714 ± 28 ppm lead) and urban particulate matter (at 6550 ± 80 ppm lead), neither of which presented a composition or matrix entirely similar to smelter soil materials around East Helena, Montana. Although the U.S. Geological Survey has documented elemental concentrations for numerous soil reference samples, none contain more than about 30 ppm of lead. Consequently, MDHES obtained Canadian (CANMET) soil and ore reference samples, the latter of which ranged from about 4 to 64% in lead. Several CANMET soils and a low-lead ore were carefully mixed, blended and ground to provide "soil" lead values within the expected range. Additional soil samples from the vicinity of the Anaconda, Montana, copper smelter, previously analyzed by EPA's NEIC laboratory in Denver by X-ray and ICP methods, were also used as calibration standards. While calibrations were nearly ideal for lead, ranging from 4 to 8310 ppm, other elements such as copper and zinc presented problems. Copper ranged from 7 to 15,800 ppm and zinc from 84 to 49,790 ppm, far greater ranges than desired for this study. For wet-chemistry analytical methods, standard solutions for elemental concentrations can easily be mixed as needed, but short of sample mixing and blending, bulk XRF standards come with fixed compositions, often with ideal concentration ranges for some elements and not for others. Standards for the garden vegetables analyzed by MDHES in this study were NBS reference materials which were both available and mostly suitable with regard to concentration ranges, although high heavy metal concentrations are lacking in NBS reference biological materials.

All ICP/AAS calibrations were set up using the EPA multiple element water standard 478-II followed by appropriate dilutions of samples, as needed, to obtain concentration values. On the other hand, X-ray calibrations utilized a suite of standards selected from reference materials where possible. A minimum of six "standards" are needed before any X-ray calibration corrections (for inter-elemental absorption and enhancements) can be made. For this study, at least a dozen "standards" were used for each calibration type to allow for needed corrections during initial instrumental calibrations.

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TABLE A

MDHES LABORATORY METHODS

	<u>Nominal Sample Size</u>	<u>Sample Treatment</u>	<u>Reference</u>	<u>Reporting</u>
Hand Wash	500 ml	None	None	ICP diskette arsenic by hand
Floor Wipe	entire sample	Extracted with Dil.Aq.Reg. in hot sonic bath	DHES Hi-vol method	ICP diskette, arsenic by hand
Vegetation	0.25 g glass bomb	HNO ₃ press.	U of Mont. Van Meter method	Hand calcula- tions
Soils (Wet-chem.)	0.200 g	Microwave with Aq.Reg. + HF	Matthes et al. U.S. Bureau of Mines	Hand calcula- tions
Soils and Vacuum Dusts (XRF)	5.000 g	Sieved @ 1 mm mesh, 0.100 g Na-sterate + 5.000 g sample to grinder; press at 16 tons/sq.in. pressure, boric acid capsuled	Modified EG&G ORTEC method	Printout to keypunch

Standard Reference Materials used for the above analyses were:

- NBS: River Sediment (1645), Fly Ash (1633), Urban Particulate (1648), Spinach (1570), Orchard Leaves (1571), Wheat (1567), Tomato (1573), Pine Needles (1575), and Bovine Liver (1577).
- USGS: AGV1, G-2 and BHVO.
- CANMET: S01, S02, PD1 (non-ferrous dust) and CZN-1 (zinc concentrate)
- USEPA: 478-II (multiple water standard), smelter soils: 4772, 4774, 4776, 4778.

TABLE B

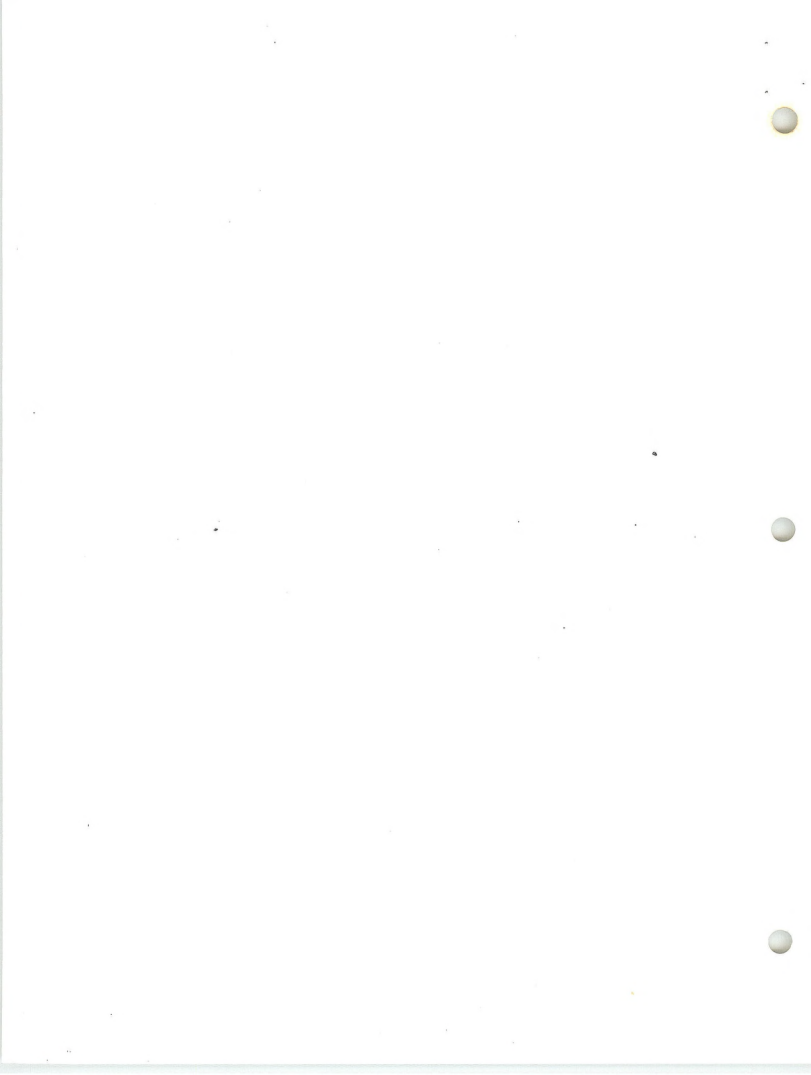
MDHES LABORATORY MINIMUM DETECTION LIMITS

Sample Type	Method	Elements							
		Al	As*	Cd	Cu	Pb	Si	Ti	Zn
Soils (ug/g - dry wt.)	ICP	5.0	0.25	2.5	2.5	12.5	25.0	2.5	2.5
Vegetation (ug/g - dry wt.)	ICP	---	0.04	0.40	0.40	2.0	---	---	0.40
Floor Wipe (ug/wipe)	ICP	0.80	0.04	0.40	0.40	2.0	---	0.40	0.04
Hand Wash (mg/l)	ICP	0.02	0.001	0.01	0.01	0.05	---	0.01	0.01
Wavelength	---	308.2	193.7	226.5	324.7	220.4	288.2	334.9	213.9
Soils/Vac (ug/g - dry wt.)	XRF	50	1.4	4.0	2.3	5.1	40	12	5.1

*Arsenic concentrations were obtained using an atomic absorption instrument equipped with hydride generation.

APPENDIX 19

312-589-0-11-0 1-REGULAR 2-CUNTRUL REF#:312- 312-589-0-21-3 *CONTACT	312-589-0-31-9 *CONSENT 312-589-0-32-0 *CONSENT#2	312-589-0-41-4 *XHF 312-589-0-42-0 *ENVIRON SAMP	312-589-0-51-A *QUEST-CHILD 312-589-0-52-1 QUEST-ADULT	312-589-0-54-5 QUEST-DIAPER 312-589-0-55-3 QUEST-HAIR
312-589-9-11-5 *DUST WIPE 312-589-9-12-5 *DUST VACUUM 312-589-9-13-7 * DUST BAG	312-589-9-21-9 *SOIL FRONT 312-589-9-22-0 *SOIL SIDE 312-589-9-23-2 * SOIL BACK	312-589-9-31-4 SOIL PLAY 312-589-9-32-0 SOIL ADJ PLAY 312-589-9-33-0 SOIL ADJ STUOL	312-589-9-41-A SOIL GARDEN 312-589-9-51-5 SOIL OTHER#1 312-589-9-52-7 SOIL OTHER#2	312-589-9-61-0 VEG #1 312-589-9-62-2 VEG# 2 312-589-9-63-4 VEG# 3
312-589-1-11-5 PERSON 1 BLOOD NAME:	312-589-2-11-2 PERSON 2 BLOOD NAME:	312-589-3-11-A PERSON 3 BLOOD NAME:	312-589-4-11-7 PERSON 4 BLOOD NAME:	312-589-5-11-4 PERSON 5 BLOOD NAME:
312-589-1-12-7 PERSON 1 BLOOD NAME:	312-589-2-12-4 PERSON 2 BLOOD NAME:	312-589-3-12-1 PERSON 3 BLOOD NAME:	312-589-4-12-9 PERSON 4 BLOOD NAME:	312-589-5-12-0 PERSON 5 BLOOD NAME:
312-589-1-21-0 PERSON 1 HANDWASHING NAME:	312-589-2-21-8 PERSON 2 HANDWASHING NAME:	312-589-3-21-5 PERSON 3 HANDWASHING NAME:	312-589-4-21-2 PERSON 4 HANDWASHING NAME:	312-589-5-21-A PERSON 5 HANDWASHING NAME:
312-589-1-31-6 PERSON 1 STUOL#1 NAME:	312-589-2-31-3 PERSON 2 STUOL#1 NAME:	312-589-3-31-0 PERSON 3 STUOL#1 NAME:	312-589-4-31-A PERSON 4 URINE (PLAIN) NAME: FILL IN PERSON#	312-589-5-31-A PERSON 5 HAIR NAME: FILL IN PERSON#
312-589-1-32-6 PERSON 1 STUOL#2 NAME:	312-589-2-32-5 PERSON 2 STUOL#2 NAME:	312-589-3-32-2 PERSON 3 STUOL#2 NAME:	312-589-4-32-A PERSON 4 URINE (ALU) NAME: FILL IN PERSON#	312-589-5-32-A PERSON 5 NAME: FILL IN PERSON#
312-589-1-33-A PERSON 1 STUOL#3	312-589-2-33-7 PERSON 2 STUOL#3	312-589-3-33-4 PERSON 3 STUOL#3	312-589-4-33-A PERSON 4 URINE (CARB) NAME: FILL IN PERSON#	312-589-5-33-A PERSON 5 NAME: FILL IN PERSON#



APPENDIX 20

ANALYTICAL METHODS AND QUALITY CONTROL ASSURANCE

FOR

EAST HELENA, MONTANA, CHILD HEALTH STUDY

Division of Environmental Health Laboratory Sciences
Center for Environmental Health
Centers for Disease Control
Atlanta, Georgia

East Helena, Montana, Child Health Study

Division of Environmental Health Laboratory Sciences

Case No. 312

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BRIEF HISTORY OF EVENT

A primary lead smelter located in East Helena, Montana, and operated by ASARCO has been in operation since 1888. A smaller, related industry, American Chemet, lies between the smelter and the city. The entire population of East Helena (1,650 persons) lies within a 1-mile radius of the smelter stack. Another 2,000 persons live within a 2-mile radius, but outside the city limits. About 5.5 square miles of the area surrounding the stack has lead exceeding 1,000 ppm in the upper 6 inches of soil.

In 1975, a random sample survey of children in East Helena found 34% of the children with blood lead levels exceeding 30 ug/dl of whole blood. 12 inches of snow was on the ground (temperature: 14°F). At the time of the study, this is significant, since the highest blood lead levels are generally found in the hottest part of the year. The testing must begin on schedule in the middle of July, if children at greatest risk for lead poisoning are to be identified.

TOXICANTS PRESENT

The principal toxicant is lead, which is probably in the form of lead oxide, due to the smelter operation. The major routes of inorganic lead absorption are the gastrointestinal tract and respiratory system. Many organs are adversely affected by lead and, often, in very complex mechanisms, such as the effects of lead on heme metabolism. Anemia is one of the early manifestations of lead exposure as the result of the shortened life span of erythrocytes. Other target organs of lead poisoning are the central nervous system, peripheral nervous system, and the kidney. Additional toxicants of interest are arsenic and cadmium that are being analyzed in environmental samples for comparative data.

PROPOSED STUDY

The Montana Department of Health and Environmental Services (DHES), in collaboration with the Division of Environmental Health Laboratory Sciences (DEHLS), is conducting a study of children in East Helena, Montana, to determine potential health risk due to lead poisoning. The DEHLS will perform analytical services to meet the goals of the study and assure the validity of biological quantitations for the study. This document presents the analytical methods/procedures and necessary quality assurances that the DEHLS will provide DHES for the Child Health Study.

LABORATORY ANALYSES REQUESTED

1. Whole Blood Lead
2. Erythrocyte Protoporphyrin
3. Whole Blood Cadmium
4. Urinary Arsenic
5. Urinary Lead/Cadmium
6. Urine B₂-Microglobulin
7. Urinary Creatinine/Protein
8. Hair Arsenic/Cadmium/Lead

INTENDED USE OF DATA

1. Validation/Confirmation

The whole blood lead and erythrocyte protoporphyrin data generated by the Division of Environmental Health Laboratory Sciences will be used to confirm and validate the respective data from ESA laboratories. A statistical comparative analysis will be performed with the data sets to determine the equivalency of the analytical results for the two laboratories.

2. Epidemiologic

All other analytes assayed by the Division of Environmental Health Laboratory Sciences are required for epidemiologic investigation of health risk related to high concentrations of heavy metals in the environment. Analytical data for these analytes will be used as specified in the study protocol.

ANALYTICAL GOALS

The analytical goals for the study set the expected minimum performance for the methods used and provide a mechanism to determine the actual laboratory performance. First, internal quality control procedures provide the past history (expected) performance of the methods and day-to-day performance by the laboratory. Second, external quality control efforts independently validate the analytical performance.

1. Internal Quality Control

A summary of the analytical goals for each analyte on the basis of the internal quality control is presented in Table 1.

2. External Quality Control

- A. A random selection of 10% of the study samples are submitted to the laboratory as "blinded" duplicate samples to determine the repeatability (sensitivity) of the analytical measurement.
- B. Blind external quality control samples are included with study specimens to independently evaluate the analytical systems for both accuracy and sensitivity.

GRAPHITE FURNACE PROCEDURE FOR BLOOD LEAD
(Revised August 18, 1983)

Determination of lead in whole blood is accomplished by flameless (electrothermal) atomic absorption. The specimen is diluted with a 0.10% v/v solution of Triton X-100, the lead homogeneously distributed throughout by lysing of the RBCs, and the absorbance of the resulting solution measured. The method is based on a published procedure by Fernandez (1,2).

EQUIPMENT

Atomic Absorption Spectrophotometer: Perkin-Elmer Model 372.

Graphite Furnace: Perkin-Elmer Model 500.

Autosampler: Perkin-Elmer Model AS-1.

<u>Parameter</u>	<u>Setting</u>
Wavelength	283.3 nm
Lamp Current	8 ma
EDL Power	9.5-10 W
Slit	0.7 (ALT)
Signal Mode	ABS
Read Time	5.0 sec
Inert Gas	Argon
Furnace Type	Pyrolytic
Background Corrector	ON
<u>Temperature Program</u>	
DRY	110 C 20 sec (20 sec RAMP)
CHAR ¹	550 C 30 sec* (10 sec RAMP)
ATOMIZE	2000 C 5 sec (1 sec RAMP)
<u>Inert Gas Flow</u>	300 mL/min 20 mL/min@ ATOMIZE

*During the CHAR portion of the temperature program, the baseline is reset to "0" with the BASELINE function of the Model 500.

¹It may be necessary to reoptimize with new contact rings or change in lots of graphite furnaces. Optimization should be checked with base (10 ug/dL) and 1.00 ppm spike (84 ug/dL) to establish:

- Peak height at maximum value.
- "Correct" value to allow calculation of base blood or other specimen
[for 84 ug/dL = $0.160A \pm 15\%$]

Recorder: Perkin-Elmer Model 56, set at 5 mV chart speed at 20 mm/min.

Automatic Pipet: Model 25000 Micromedic Automatic Pipette, equipped with a 1-mL dispensing pump (set at 100%) and a 200-uL sampling pump (set at 50%).

NOTE: A 1-mL sampling pump set at 10% has also been found satisfactory.

REAGENTS

Water: Ultrapure water, prepared by a Milli-Q polishing system, is used throughout.

Nitric Acid: Redistilled nitric acid (G. Frederick Smith) is used to prepare a 1.0% v/v dilution by volumetric dilution with ultrapure water.

Triton X-100: Scintillation Grade (Eastman Kodak) is used to prepare 0.10% v/v solution by volumetric dilution with ultrapure water.

Stock and Working Lead Standards: A 1,000 mg/L stock solution of lead nitrate is prepared from 1.5985 g of NBS SRM 928 lead nitrate, diluted to 1L with 1.0% v/v nitric acid. This solution is prepared every 6 months. Intermediate standard of 10 mg/L and working standards of 0.10, 0.25, 0.50, 0.75 and 1.00 mg/L are prepared daily by volumetric dilution with ultrapure water.

PLASTICWARE AND GLASSWARE

All plasticware and glassware used is cleaned by soaking 24 h in detergent, followed by soaking for 3 days in 25% v/v nitric acid. The cleaned items are then rinsed thoroughly with ultrapure water, and stored in a dust-free environment.

Venous blood specimens are collected in either 5-mL Vacutainers (Beckton and Dickinson) or 5-mL "Monoject" tubes (Sherwood Manuf). Both of these containers employ aqueous dipotassium EDTA as anticoagulant.

Eppendorf micropipets (Brinkmann Instruments) are used to prepare spiked aliquots for calibration; disposable polyethylene tips are used as received.

Beckmann Instruments' "Bio-Vials" are used for dilution of blood specimens; these 4-mL containers are cleaned as above.

SPECIMEN COLLECTION

Collection of an uncontaminated whole blood specimen is a critical part of many toxicological investigations. The following guidelines will provide directions which, if carefully followed, will minimize the contamination of whole blood by the many sources from which it may come. It cannot be overemphasized that skin, clothing, dust, and many other sources of contamination contain many times the levels of lead, arsenic, cadmium, and other metals that will be determined in the collected specimen.

1. Clean the antecubital area thoroughly with: a) soap and water (Phisoex has been shown to be free from significant metal contamination), followed by b) alcohol (isopropanol or ethanol).
2. Puncture the skin/vein with a sterile, disposable needle capable of multiple sampling. A suggested product is the B + D catalog #5749, 20-gauge needle. In some applications, the first blood specimen collected will be used for other determinations; its use is to "rinse" the collection needle with blood.
3. Collect one or more tubes of whole blood, using an appropriate anticoagulant for the metal of interest. Anticoagulant/metal combinations that have been shown to be compatible are:

Mercury-Heparin or Citrate
Lead-EDTA, Heparin, or Oxalate
Cadmium-EDTA, Heparin or Oxalate
(preferred anticoagulant underlined)

It is critical that a few "spares" of the lot of tubes used for collection be sent to the laboratory along with the collected specimens. This will

- allow the laboratory to determine the metal content of the anticoagulant used in that tube lot and to make appropriate blank corrections. Of course, all lots of tubes should be screened before use in surveys.
4. It is critical that the collected specimen be thoroughly mixed after collection, to insure the anticoagulant/blood mixture is uniform and that clotting therefore will be prevented. Clotted specimens are nearly useless!
 5. Refrigerate the collected specimens, and ship them refrigerated by the most expeditious means available. Heparin is by far the least "permanent" of the anticoagulants listed, but it will prevent clotting for 2 weeks if well-mixed during collection and if the specimens are refrigerated after collection and during shipment.
 6. Ship the collected specimens in well-padded, insulated containers (freeze safe or the equivalent).

ANALYTICAL PROCEDURE

1. Aspirate 100 μ L of blood into the delivery tip of the automatic pipet and dispense sample and 1.00 mL of 0.10% v/v Triton X-100 into a precleaned 4mL plastic vial.
2. Aspirate air into the delivery tip and dispense 1.00 mL of 0.10% v/v Triton X-100 into the same vial. Cap the diluted specimen with a plastic top.
3. Vortex thoroughly, and allow the diluted specimen to stand 15-20 minutes.
4. Re-vortex, and transfer a portion of the diluted specimen to a precleaned polyethylene or polypropylene autosampler cup.
5. Measure the absorbance in triplicate, using the AS-1 autosampler to dispense 20 μ L of diluted specimen into the graphite furnace.

STANDARDIZATION AND CALCULATIONS

Standardization is accomplished by the use of a modification of the method of standard additions. A bovine "base blood," typically containing less than 10 μ g/dL of endogenous lead, is diluted as in the procedure, and aliquots are spiked with microliter additions of lead nitrate standards.

Standard Addition Procedure:

1. Base blood is diluted in the procedure, using twice the prescribed volume (perform a "double" dilution).
2. Into five precleaned plastic autosampler cups, pipet 20 μ L of 0.10, 0.25, 0.50, 0.75, and 1.00 mg/L lead nitrate, using a 20- μ L Eppendorf micropipet with disposable plastic tips.
3. Add 500 μ L of diluted base blood to each of the five cups, using a 500- μ L Eppendorf with plastic tips. Allow the diluted specimen to drain in the plastic tip, and apply several more displacement strokes to assure a quantitative transfer. Mix by gentle swirling.

NOTE: Careful measurement by gravimetric methods have indicated that the transfer of diluted specimens is quantitative, provided careful technique is used.

4. Transfer the remaining diluted base blood to a sixth autosampler cup.
5. Measure the absorbances in triplicate as in the procedure.

Calculations:

Two methods of calculation have been used with the described procedure, each of which gives essentially identical results. Each individual specimen may be analyzed by the procedure outlined above for standard additions. Although potentially highly accurate, this method has an extremely low throughput. Any differences in observed slope from spiked aliquots of different specimens will be automatically compensated for by this method. Since no measurable difference in slope was observed (within experimental error) between spiked bovine and human blood, either a regression analysis or an "average slope" method are normally used.

Method 1

Prepare spiked aliquots of all specimens, measuring absorbances in triplicate. The average of three absorbances of the six aliquots are taken to be:

- A = average absorbance of unspiked specimen
- B = average absorbance of first spike (+ 2 ng)
- C = average absorbance of second spike (+ 5 ng)
- D = average absorbance of third spike (+ 10 ng)
- E = average absorbance of fourth spike (+ 15 ng)
- F = average absorbance of fifth spike (+ 20 ng)

All spiked average absorbances are corrected for dilution, multiplying by a factor df, where:

$$df = \text{dilution factor} = \frac{\text{orig. volume} + \text{spike volume}}{\text{orig. volume}}$$

In this case, with a 0.500-mL original volume and a 0.020-mL spike volume, the factor is:

$$\frac{(0.500 + 0.020)}{0.500} \text{ mL} = \frac{0.520}{0.500} \text{ mL} = 1.04$$

Calculations1. Linear regression

Calculate mean of 2 or 3 measured absorbances for base and 5 standards.
Correct "standard" absorbances by the dilution factor 1.04.

Construct the following table:

Added mg/dL	Blood	Average absorbance	A corrected	$(A \text{ corr} - A_{\text{base}}) = N(1-5)$
0	Base	A_{base}	-	0
8.4	0.10 ppm spike	A_1	$A_1(1.04)$	N_1
21.0	0.25 ppm spike	A_2	$A_2(1.04)$	N_2
42.0	0.50 ppm spike	A_3	$A_3(1.04)$	N_3
63.0	0.75 ppm spike	A_4	$A_4(1.04)$	N_4
84.0	1.00 ppm spike	A_5	$A_5(1.04)$	N_5

Using a calculator, enter the following:
 ug/dL, A corr -Abase

(0	,	0)
(8.4	,	N ₁)
(21.0	,	N ₂)
(42.0	,	N ₃)
(63.0	,	N ₄)
(84.0	,	N ₅)

Calculate r^2 slope, intercept. Typical values for these parameters are:
 slope 1.8 - 2.2
 intercept -1.0 - +1.0
 r^2 0.98

- Using corrected (A - blank) specimen values, calculate ug/dL for unknowns.
2. Average slope
 Using values in table above, calculate the following:
- | | | | |
|-------------|-------------|-------------|-------------|
| \bar{N}_2 | \bar{N}_3 | \bar{N}_4 | \bar{N}_5 |
| 2.5 | 5.0 | 7.5 | 10.0 |

Average these values and calculate a conversion factor, cf:

$$cf = \frac{8.4 \text{ ug/dL}}{\bar{N}(1-5)}$$

Where $\bar{N}(1-5)$ = average of N_1 and four calculated values above.

Take corrected A values (- blank) for specimens, and calculate ug/dL by multiplying by cf.

NOTE: [cf should be 0.45 - 0.55]

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two-way analysis of variance, ANOVA, with the construction of quality control charts based on 95% and 99% confidence limits of the mean of duplicate measurements, as well as range charts (3).

Precision and accuracy of the analytical system will be monitored as follows:

- 1) Ten analytical runs will be performed to characterize all control materials used, with duplicate measurements performed per run.
- 2) Analysis of variance calculations will be performed on these 20 data points, and quality control charts will be generated by computer for X and range R.
- 3) A minimum of two control materials will be incorporated into each analytical run of 20 unknown specimens, and data obtained for these controls will be evaluated with the X and R charts from 2).

Blind Quality Control

Two types of blind quality control specimens will be incorporated into the system:

- 1) blind duplicate specimens will be prepared, and inserted at an interval determined by the supervisor, usually one blind duplicate per 20 unknown specimens.
- 2) blinded control or reference material samples will be inserted into each analytical run of 20 specimens, at the minimum rate of one blind control per 20 specimens.

In both cases, the blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Run Format

In all cases, the following format will be used for specimen determination:

<u>SAMPLE #</u>	<u>Sample ID</u>
1	Blank
2-5 (or greater)	Calibration Curve
6	Control I
7-27	Specimens
28	Control II
29-33	Calibration Curve
34	Blank

Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (3):

X chart

- 1) A single X value falls above the upper 99% limit or below the lower 99% limit.
- 2) Two successive X values fall either both above the upper 95% limit or both below the lower 95% limit.
- 3) Eight X values in succession fall either all above the center line or all below the center line.

R chart

- 1) A single R value falls above the upper 99% limit.
- 2) Two successive R values fall above the 95% upper limit.
- 3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

- 1) Check for errors in recording levels of control samples, and if none are found,
- 2) Check and calibrate instruments before performing further analyses on analytical samples,
- 3) Reanalyze patient samples performed during the out-of-control run.

Method Performance

Limits of Detection

Limit of detection is in a practical sense determined by two factors: 1) the slope sensitivity of the method, i.e., the response (in the case of atomic absorption, the absorbance or absorbance-second measurement) for a given concentration or amount of analyte, and 2) the random noise of the instrumental system used in measurement, especially that noise at the measured response for the blank for the determination (4).

According to the recommendations of the above reference, the limit of detection will be defined as that concentration of analyte corresponding to an absorbance or absorbance-second measurement equivalent to three times the standard deviation of this signal measured at an analyte concentration at a "low" level. In symbolic terms, this becomes:

$$c_L = \frac{3s_B}{m}$$

where c_L is the concentration calculated to be the limit of detection, s_B is the standard deviation of the measurement of a blank or low concentration sample; and m is the slope of the calibration curve (change in absorbance or absorbance-seconds/change in concentration or amount of analyte). For a set of measurements at the 12-ug/dL level, the standard deviation of the measurement was $1.33 \times 10^{-3}A$, which yields a limit of detection of 2.04 ug/dL ($N = 6$).

Accuracy and Precision

Accuracy and precision of the blood lead method have been estimated from the determination of whole blood pools whose target values have been determined by either a definitive method (5) or by a series of highly proficient reference laboratories. The accuracy and precision of this method have been presented in a previous publication.

Recent evaluation of the revised method was performed with the use of whole blood pools whose target or "true" values were determined by the National Bureau of Standards by stable isotope dilution-mass spectroscopy. Precision at the "medical decision" level of 30 ug/dL was measured to be 6% or less (% total CV) with accuracy of ± 2 ug/dL or less versus ID-MS target values. The accuracy and precision shown by these data are comparable to that obtained in previously published work (6).

References

1. Fernandez FJ. Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace, Clin Chem 21 (4): 558-561 (1975).
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5. "A national understanding for development of reference methods and materials for clinical chemistry. J.H. Boutwell, ed., American Association of Clinical Chemistry, Washington, D.C., 1978.
6. Paschal D and Bell C Improved accuracy in determination of blood lead by electrothermal atomic absorption. Atomic Spectroscopy, 2: 146-50 (1981).

ERYTHROCYTE PROTOPORPHYRIN

I. Principle

Free erythrocyte protoporphyrin (FEP) is measured by a modification of the method of Sassa and Granick et al.(1) Protoporphyrin is extracted from EDTA-whole blood into a 2:1 (v/v) mixture of ethyl acetate-acetic acid, then back-extracted into dilute hydrochloric acid. The protoporphyrin in the aqueous phase is measured fluorometrically at excitation and emission wavelengths of 404 and 655 nm, respectively. Calculations are based on a processed protoporphyrin IX (free acid) standard curve. The final concentration of protoporphyrin in a specimen is expressed as micrograms per deciliter of packed red blood cells (ug/dL) RBCs; a correction for the individual hematocrit is made.

II. Instrumentation

- A. Perkin-Elmer Model 650-10 spectrofluorometer, with R938 photomultiplier tube, xenon lamp, and custom-made microcell (10- X 75-mm) holder positioned to allow the passage of light through the aqueous phase only.
(Perkin-Elmer Corp., Norwalk, CT)
- B. Model 56 recorder
(Perkin-Elmer Corp.)
- C. Cary Model 119 double-beam spectrophotometer
(Varian Associates, Palo Alto, CA)
- D. Vortex mixer
(Fisher Scientific Co., Fairlawn, NJ)
- E. Micromedic APS-2 pipetting station with 50-uL sampling and 200-uL dispensing pumps and reagent dispenser, with inserts in racks modified to accept 10- X 75-mm tubes.
(Micromedic Systems, Division of Rohm and Haas, Horsham, PA)
- F. Mettler Model H18 analytical balance
(Mettler Instrument Corp., Hightstown, NJ)
- G. IEC Centra-7 centrifuge
(International Centrifuge Co., Needham Heights, MA)
- H. Hamilton Dispenser, with 2.5-mL syringe
(Hamilton Co., Reno, NE)
- I. Micromedic High-Speed Automatic Diluter with 1.0-mL dispensing pump
(Micromedic Systems)

III. Materials

- A. Protoporphyrin IX, dimethyl ester, 99.3% purity, grade 1 (Sigma Chemical Co., St. Louis, MO)
NOTE: Store at -20°C over a desiccant. Purchase of one lot is recommended.
- B. Ethyl acetate, spectrophotometric quality
(J. T. Baker Co., Phillipsburg, NJ)
- C. Acetic acid, glacial, "Baker Analyzed"
(J. T. Baker Co.)
- D. Hydrochloric acid, concentrated, "Baker Analyzed"
(J. T. Baker Co.)
- E. Kimble 10- X 75-mm disposable glass culture tubes
(Kimble Div., Owens-Illinois Co., Toledo, OH)
- F. Parafilm M
(American Can Co., Greenwich, CT)

- G. Actinic glass volumetric flasks
(Corning Glassworks, Corning, NY)
NOTE: All nondisposable glassware used in this assay should be washed in 10% (v/v) nitric acid and rinsed six times with deionized water.
- H. Formic acid, 88%, reagent grade
(J. T. Baker Co.)
- I. Deionized water, greater than or equal to 1.0 megaOhm-cm at 25°C
(Continental Water Co., Atlanta, GA)

IV. Reagent Preparation

- A. 7.0 mol/L Hydrochloric acid (HCl) (for hydrolysis)
Dilute 551 mL concentrated HCl to volume with deionized water in a 1-liter volumetric flask.
- B. 1.79 mol/L HCl (for daily absorbance readings)
Dilute 141 mL concentrated HCl to volume with deionized water in a 1-liter volumetric flask.
- C. 0.43 mol/L HCl (for analysis-extraction)
Dilute 68 mL concentrated HCl to volume with deionized water in a 2-liter volumetric flask.
- D. 1.5 mol/L HCl (for blanking spectrophotometer)
Dilute 118 mL concentrated HCl to volume with deionized water in a 1-liter volumetric flask.
NOTE: These dilutions assume concentrated HCl to be 12.7 mol/L. The molar concentration of different lots of HCl should be calculated by using the following formula:

$$\text{mol/L} = \frac{\text{relative density} \times \% \text{HCl}}{35.453}$$

- E. 2:1 (v/v) Ethyl acetate - acetic acid
Working under a hood, combine 200 mL ethyl acetate and 100 mL glacial acetic acid. Mix the solution well; this volume is sufficient for the standards, controls, and 80 specimens. (Prepare this reagent daily, immediately before sampling the whole blood.)

V. Standard Preparation

NOTE: Prepare all standard solutions in actinic glass volumetric flasks.

- A. Protoporphyrin IX Standards (Concentrations are expressed in terms of protoporphyrin IX free acid after the dimethyl ester has been hydrolyzed. The millimolar absorptivity of protoporphyrin IX free acid has conventionally been determined in 1.5 mol/L HCl; thus, the daily absorbance reading of the hydrolysate is determined at this acid concentration.(2))
 - 1. 20 mg/dL Protoporphyrin IX Free Acid Hydrolysate (Stock Standard)
Measure 42.0 mg protoporphyrin IX dimethyl ester (PPIX DME).

Dilute to volume in a 200-mL actinic volumetric flask with 7 mol/L HCl, washing PPIX off weighing paper with a few drops of formic acid. Add a small stirring bar, cover the flask with aluminum foil, and mix contents at 20-25°C for 3 h, using a magnetic stirrer. (Prepare weekly.)

2. 1,000 ug/dL Intermediate Stock

After 3 h, dilute 25.0 mL of 20 mg/dL solution with deionized water to volume in a 500-mL actinic volumetric flask to yield a 1000 ug/dL solution, which is 0.35 mol/L with respect to HCl. (Prepare weekly.)

3. 100 ug/dL Standard for Daily Absorbance Readings

Dilute 10.0 mL of 1,000 ug/dL intermediate stock to volume in a 100-mL actinic volumetric flask with 1.79 mol/L HCl to yield a 100 ug/dL protoporphyrin IX standard, which is 1.5 mol/L with respect to HCl. Use an aliquot of this standard for absorbance readings, as in Section VI.B.

NOTE: The theoretical concentration of this solution with respect to protoporphyrin IX free acid (PPIX FA) is calculated as follows:

$$\frac{42 \text{ mg PPIX DME}}{200 \text{ mL}} \times \frac{562.27 \text{ mg PPIX FA}}{590.72 \text{ mg PPIX DME}} = \frac{.1999 \text{ mg PPIX FA}}{\text{mL}}$$

$$\frac{.1999 \text{ mg PPIX FA}}{\text{mL}} \times \frac{25 \text{ mL}}{500 \text{ mL}} \times \frac{10 \text{ mL}}{100 \text{ mL}} = .0009975 \text{ mg/mL PPIX FA (99.75 ug/dL)}$$

$$\frac{99.95 \text{ ug}}{1 \text{ dL}} \times \frac{1 \text{ mmol}}{562.27} \times \frac{10 \text{ dL}}{1 \text{ L}} \times \frac{1 \text{ mg}}{1000 \text{ ug}} = .00178 \text{ mmol/L PPIX FA}$$

4. 100 ug/dL Standard for Dilutions

Dilute 5.0 mL of 1,000 ug/dL intermediate stock to volume with 0.43 mol/L HCl in a 50-mL actinic volumetric flask.

5. 0-80 ug/dL Working Standards

Prepare the following working standards daily by diluting the 100 ug/dL standard with 0.43 mol/L HCl according to the following dilution scheme, using a Micromedic APS-2 equipped with 50-uL sampling and 200-uL dispensing pumps, and the reagent dispenser.

<u>Working Standard Concentration</u>	<u>Volume 1,000 ug/dL Standard</u>	<u>Volume 0.43 mol/L HCl Diluent</u>	<u>Final Volume</u>
80 ug/dL	400 uL	4,600 uL	5,000 uL
70 ug/dL	350 uL	4,650 uL	5,000 uL
60 ug/dL	300 uL	4,700 uL	5,000 uL
50 ug/dL	250 uL	4,750 uL	5,000 uL
40 ug/dL	200 uL	4,800 uL	5,000 uL
30 ug/dL	150 uL	4,850 uL	5,000 uL
20 ug/dL	100 uL	4,900 uL	5,000 uL
10 ug/dL	50 uL	4,950 uL	5,000 uL
0 ug/dL	0 uL	5,000 uL	5,000 uL

NOTE: It is especially important to work under subdued lights when diluting and extracting the standard materials, which are photo-labile.

VI. Procedure

NOTE: To protect hands against acids and solvents during sampling, wear latex gloves. To avoid evaporation or degradation of specimens, process samples as rapidly as possible. After centrifugation, samples are stable for 1-3 h.

- A. Thaw specimens and quality control materials of frozen EDTA-whole blood at room temperature.

NOTE: Control pools with elevated levels of FEP are prepared from blood (EDTA-anticoagulated) collected from cows that have been administered lead acetate.

- B. Using the spectrophotometer and quartz cuvettes, measure absorbance at wavelength-maximum of the 100 ug/dL in 1.5 mol/L HCl standard solution against a blank of 1.5 mol/L HCl, scanning from 380-420 nm. (Wavelength-maximum is about 407-408 nm.) This measurement will be used to determine standard concentrations. Clean cuvettes after use with 5% Contrad solution, rinse thoroughly with deionized water, followed by ethanol to remove water droplets.
- C. Prepare the working standard dilutions from 100 ug/dL standard in 0.43 mol/L HCl, using 0.43 mol/L HCl as a diluent. These dilutions are unstable; therefore, prepare them as rapidly as possible.
- D. Prepare the 2:1 ethyl acetate-acetic acid mixture and fill the dispenser bottle of the Micromedic high-speed dilutor for delivering 1.0 mL of reagent. Fill the dispenser bottle of the Hamilton Dilutor with 0.43 mol/L HCl for delivery of 1.0 mL. (Place dilutors under hood to minimize fumes during usage.)
- E. Before sampling, vortex thoroughly each standard dilution, quality control pool, or whole blood specimen. Using the APS-2, transfer 10 uL of sample to a 10- X 75-mm disposable glass tube, in duplicate.
- F. Add 1.0 mL of the 2:1 ethyl acetate-acetic acid mixture to each sample. Vortex thoroughly for 10 sec.
- G. Add 1.0 mL of the 0.43 mol/L HCl to each sample. Wrap tube with Parafilm and vortex thoroughly for 10 sec.
- H. Sample in this order: standards, quality control pools, and whole blood specimens in duplicate.
- I. Prepare two blank tubes (0 standards) with 1.0 mL each of ethyl acetate-acetic acid and 0.43 mol/L HCL, with 10 ul 0.43 HCL as sample.
- J. When all sampling is completed, centrifuge all tubes for 4 min at 1,400 rpm.
- K. For samples outside the range of the standard curve, use a smaller sample size or dilute sample with saline. For example,
5 uL = 1:2 dilution
2 uL = 1:5 dilution
100 uL sample and 900 uL saline = 1:10 dilution, 10 uL sample used

L. Perkin-Elmer 650-10 spectrofluorometer settings

slit(s) width	10 nm
photomultiplier tube	R938 Hamamatsu
cuvettes	10- X 75-mm in microcell adapter
range	1
PM Gain	normal
response	normal
mode	normal
scan	off
wavelength	404 nm excitation
	655 nm emission

- M. Allow 30-45 min for the 650-10 to warm up and stabilize after the xenon lamp has been ignited.
- N. Following the instruction manual, zero the Model 56 recorder with "Recorder Zero" and "MEAS."
- O. With shutter closed and sensitivity set on "1," zero the 650-10 spectrofluorometer using "Zero Adjust" with "Zero Suppression" OFF.
- P. Open shutter. Turn "Zero Suppression" on. Put tube with blank solution in sample compartment, and zero the digital readout carefully using the zero suppression knob.
- NOTE: Because of tube-to-tube variance, it is important to check several different blank tubes and take the average amount of the blank to be zeroed out.
- Q. Place 80 ug/dL PPIX standard tube in sample compartment and set digital readout to 80.0, using "sensitivity fine" knob. Check with another 80 ug/dL standard to verify.
- R. Proceed to read the standard curve, quality control pools, and samples.

VII. Calculations

The millimolar absorptivity of protoporphyrin IX free acid in 1.5 mol/L HCl has been determined in our laboratory to be $296.87 \pm .45$ (400 observations). Calculate the actual concentration of the 100 ug/dL (.00178 mmol/L) working standard, using the following equation:

$$A = ebc, \text{ and } c = \frac{A}{eb}$$

Where:

- A = absorbance reading
 b = cuvette pathlength, 1 cm
 c = concentration, in mmol/L
 e = millimolar absorptivity of protoporphyrin IX free acid in 1.5 mol/L HCl, 296.87

For example, if the daily absorbance reading of the 100 ug/dL standard at wavelength maximum is 0.520, then:

$$C = \frac{0.520}{(297 \text{ L/mmol-cm}) (1 \text{ cm})} = .00175 \text{ mmol/L}$$

Then: $(.00175 \text{ mmol/L}) (562.27 \text{ mg/mmol}) (1000 \text{ ug/mg}) (1\text{L}/10 \text{ dL}) = 98.40 \text{ ug/dL}$

PPIX FA

Consider 98.40 as a percentage of 100 ug/dL and correct the standard curve accordingly:

$$10 \text{ ug/dL} \times .9840 = 9.84$$

$$20 \text{ ug/dL} \times .9840 = 19.68, \text{ etc.}$$

Perform a linear regression, with x = corrected standard concentration and y = fluorescent intensity reading. Using the slope of the standard curve and assuming zero intercept, calculate the concentration of protoporphyrin IX per deciliter of whole blood for each specimen. To correct for hematocrit and express results as ug/dL of RBC, use this formula:

$$\frac{\text{ug/dL whole blood}}{\text{hematocrit}} \times 100 = \text{ug/dL RBC}$$

VIII. CDC Modifications

The following modifications of the original methods are based on CDC optimization experiments: (a) sample size increased from 2 uL to 10 uL; (b) ethyl acetate-acetic acid and 0.43 mol/L HCl volumes increased from 0.3 mL to 1.0 mL; (c) processed protoporphyrin IX standards used; (d) hydrolysis time for the dimethyl ester decreased from 48 h to 3 h, on the basis of the work of Culbreth et al.(3); and (e) 0.43 mol/L HCl chosen for maximum fluorescent intensity of the extracted

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two-way analysis of variance, ANOVA, with the construction of quality control charts based on 95% and 99% confidence limits of the mean of duplicate measurements, as well as range charts (1).

Precision and accuracy of the analytical system will be monitored as follows:

- 1) Twenty analytical runs will be performed to characterize all control materials used, with quadruplicate measurements performed per run.
- 2) Analysis of variance calculations will be performed on these 20 runs, and quality control charts will be generated by computer for \bar{X} and range R .
- 3) Three levels of quality control materials will be incorporated into each day's analytical run, and data obtained for these controls will be evaluated with the \bar{X} and R charts from 2).

Blind Quality Control

Two levels of blind quality control pools will be incorporated into the system:

Blind duplicate specimens will be prepared and inserted at an interval determined by the supervisor, usually one blind duplicate per 20 unknown specimens.

The blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (1):

X chart

- 1) A single X value falls above the upper 99% limit or below the lower 99% limit.
- 2) Two successive X values fall either both above the upper 95% limit or both below the lower 95% limit.
- 3) Eight X values in succession fall either all above the center line or all below the center line.

R chart

- 1) A single R value falls above the upper 99% limit.
- 2) Two successive R values fall above the 95% upper limit.
- 3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

- 1) Check for errors in recording levels of control samples, and if none are found,
- 2) Check and calibrate instruments before performing further analyses on analytical samples,
- 3) Reanalyze patient samples performed during the out-of-control run.

IX. References

1. Sassa S, Granick JL, Granick S, Kappas A, and Levere RD: Microanalyses of erythrocyte protoporphyrin levels by spectrophotometry in the detection of chronic lead intoxication in the subclinical range. *Biochem Med* 8:135-148 (1973).
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GRAPHITE FURNACE PROCEDURE FOR BLOOD CADMIUM

(Revised August 26, 1983)

Determination of blood cadmium is accomplished by flameless (electrothermal) atomic absorption. The specimen is deproteinized with the addition of nitric acid after dilution with water, and cadmium is determined in the acid supernatant. A L'vov platform is used to decrease matrix effects (1) and to increase precision and sensitivity (2). The procedure is based on published work by Stoeppler et al. (3).

EQUIPMENT

Atomic Absorption Spectrophotometer: Perkin Elmer Model 372, with Model 500 graphite furnace, Model AS-1 autosampler, and Model 56 recorder. Pyrolytic graphite furnaces and L'vov platforms are used.

Instrument Parameters:

<u>Parameter</u>	<u>Setting</u>
Wavelength	228.8 nm
Lamp Current	6 ma
EDL Power	6 W
Slit	0.7 (alt)
Signal Model	ABS
Read Time	4.0 s
Inert Gas	Argon
Furnace Type	Pyrolytic/L'vov
Background Corrector	ON

Temperature Program

DRY	150 C 25 s (5 s ramp)
CHAR	450 C 25 s (5 s ramp)*
ATOMIZE	2000 C 5 s (1 s ramp)
COOL	20 C 10 s (1 s ramp)
Inert Gas Flow	300 mL/min; 20 mL/min @ ATOMIZE

*During the latter part of the CHAR, the baseline is reset to "0" by using the BOC function of the 372; the settings at step 2 (char) are: READ 20s; BASELINE 28s; REC 29s. READ and REC are set "0" during ATOMIZE.

Recorder: Model 56 set at 5 mV; 20 mm/min speed.

Automatic Pipet: Micromedic Model 25000, with 1-mL sampling and dispensing pumps; sampling pump set at "20%" (200 uL), dispensing at "50%" (500 uL).

REAGENTS

Water: Ultrapure water, polished by a Milli-Q system to 18 megaohm/cm purity is used throughout.

Nitric Acid: Redistilled grade nitric (GF Smith) or Ultrex (JT Baker) is used.

Stock and Working Cadmium Standards: A 1,000 mg/L stock solution of reagent grade cadmium acetate dihydrate is prepared from 237 mg of the cadmium salt, dissolved and diluted to 100 mL with ultrapure water. To a 100-mL volumetric flask, add 237 mg of the salt and 500 uL ultrapure nitric acid, diluting to

the mark with ultrapure water. This stock solution should be prepared every 6 months. Intermediate stock of 10 mg/L is prepared by 1:100 volumetric dilution of the 1,000 mg/L solution weekly; working and spiking stocks of 1.0 mg/L, 10, 25, 50, and 75 ng/mL are prepared daily from the 10 mg/L intermediate stock.

PLASTICWARE AND GLASSWARE

All plasticware and glassware used is cleaned by soaking 24 h in detergent, followed by soaking for 3 days in 25% v/v nitric acid. The cleaned items are then rinsed thoroughly with ultrapure water, dried under class 100 conditions, and stored in a dust-free environment.

Venous blood specimens are collected in either 5-mL vacutainers (Beckton and Dickinson) or 5-mL "Monoject" tubes (Sherwood Manuf.). Both containers employ aqueous dipotassium EDTA as anticoagulant.

Eppendorf micropipets (Brinkmann Instruments) are used to prepare spiked aliquots for calibration; disposable polyethylene tips are used as received.

Beckmann Instruments' "Bio-Vials" are used for dilution of blood specimens; these 4-mL containers are cleaned as above.

SPECIMEN COLLECTION

Collection of an uncontaminated whole blood specimen is a critical part of many toxicological investigations. The following guidelines will provide directions which, if carefully followed, will minimize the contamination of whole blood by the many sources from which it may come. It cannot be overemphasized that skin, clothing, dust, and many other sources of contamination contain many times the levels of lead, arsenic, cadmium, and other metals that will be determined in the collected specimen.

1. Clean the antecubital area thoroughly with: a) soap and water (Phisohex has been shown to be free from significant metal contamination); followed by b) alcohol (isopropanol or ethanol).
2. Puncture the skin/vein with a sterile, disposable needle capable of multiple sampling. A suggested product is the B + D catalog #5749, 20-gauge needle. In some applications, the first blood specimen collected will be used for other determinations; its use is to "rinse" the collection needle with blood.
3. Collect one or more tubes of whole blood, using an appropriate anticoagulant for the metal of interest. Anticoagulant/metal combinations that have been shown to be compatible are:

Mercury-Heparin or Citrate
Lead-EDTA, Heparin, or Oxalate
Cadmium-EDTA, Heparin, or Oxalate
(preferred anticoagulant underlined)

It is important that a few "spares" of the lot of tubes used for collection be sent to the laboratory along with collected specimens. This will allow the laboratory to determine the metal content of the anticoagulant used in that tube lot and make appropriate blank corrections. Of course, all lots of tubes used should be screened before use in surveys.

4. It is critical that the specimen be thoroughly mixed after it has been collected to insure that the anticoagulant/blood mixture is uniform and that clotting will therefore be prevented. Clotted specimens are nearly useless!

5. Refrigerate the collected specimens, and ship refrigerated by the most expeditious means available. Heparin is by far the least "permanent" of the anticoagulants listed, but it will prevent clotting for 2 weeks if the specimen and anticoagulant are well-mixed at the time of collection and refrigerated after collection and during shipment.
6. Ship the collected specimens in well-padded, insulated containers (freeze safe or the equivalent).

ANALYTICAL PROCEDURE

1. Aspirate 200 μ L of blood into the delivery tip of the automatic pipet; dispense blood and 500 μ L water into a precleaned 4-mL plastic vial.
2. Aspirate air into the delivery tip, and dispense an additional 500 μ L water into the same vial. This "double rinse" should give a quantitative transfer of blood to the plastic vial.
3. Add 50 μ L of ultrapure nitric acid to the diluted specimen, cap and mix thoroughly on a vortex-type mixer until protein precipitation is complete, as evidenced by the color of the solution changing to a dark brownish red and the dispersion of precipitated protein/RBC's throughout the diluted specimen.
4. Centrifuge at 2,000 RPM for 5 minutes.
5. Decant the acid supernatant with a 250- or 500- μ L Eppendorf pipet.
6. Measure the absorbance of the resulting supernatant in duplicate or triplicate, using the AS-1 autosampler to dispense 20 μ L of solution into the graphite furnace.

STANDARDIZATION AND CALCULATIONS

Standardization is accomplished by the use of a modification of the method of standard additions. A bovine "base blood," typically containing less than 1 ng/mL cadmium, is diluted per the procedure, and aliquots are spiked with microliter additions of cadmium acetate standards.

Standard Addition Procedure:

1. Base (low cadmium) blood is prepared per analytical procedure, step 1-5, using twice the prescribed volumes.
2. Into four precleaned autosampler cups, pipet 10 μ L of 10, 25, 50, and 75 ng/mL cadmium standard, using a 10- μ L Eppendorf pipet with disposable plastic tips.
3. Add 250 μ L of acid supernatant prepared from "base" blood to each of the four cups.
4. Transfer 250 μ L of the remaining supernatant to a fifth autosampler cup.
5. Measure the absorbance of the resulting solutions in duplicate or triplicate.

Calculations:

Two methods of calculation have been used with the described procedure, each of which give essentially identical results. Each individual specimen may be analyzed by the procedure outlined above for standard additions. Although potentially highly accurate, this method suffers from extremely low

throughput. Any differences in observed slope from spiked aliquots of different specimens will be automatically compensated for by this method. Since no measurable difference in slope was observed (within experimental error) between spiked bovine and human blood, either regression analysis, Method 2, or an "average slope" method, Method 1, are normally used.

Method 1 Average Slope

1. Calculate the average (mean) absorbance values for the solutions measured.
2. Correct the mean absorbances of the cadmium-spiked solutions by a dilution factor, df, calculated as follows:

$$df = \frac{\text{original volume} + \text{spiking volume}}{\text{original volume}}$$

In this procedure, the df will be 1.04, which is

$$\frac{250 \text{ uL} + 10 \text{ uL}}{250 \text{ uL}} + \frac{260 \text{ uL}}{250 \text{ uL}} = 1.04$$

3. Construct the following table:

Specimen	Corrected Absorbance	ng/mL added	Corrected Absorbance - A _{base}
Base	A _{base}	0	0
Spike ₁	A (1.04) spike 1	2.5	A (1.04) - A _{base} spike 1
etc.			

4. Calculate the factor, (ng/mL added)/(corrected absorbance - A_{base}) for each of the four spiked calibrators. The ng/mL additions are calculated as additions to the original blood specimen and have the values 2.5, 6.25, 12.5, and 18.75 ng/mL.
5. Average the four factors from step 4 and multiply specimen absorbances (controls or unknowns) by this average factor to calculate the cadmium content in ng/mL. Make sure that all specimen absorbances are corrected for blanks (dilute nitric acid) before calculation.

NOTE: The value for the average of (ng/mL)/(corrected absorbance - A_{base}) for this procedure usually falls in the range of 0.05-0.07 for the 2.5-ng/mL addition. The calculated factors should be examined for consistency; this is one indication of linearity of the calibration curve.

Method 2-Linear Regression

1. Construct the following table:

X (ng/mL added)	Y ($A_{\text{corrected}} - A_{\text{base}}$)
0	0
2.5	$A(1.04) - A_{\text{base}}$ spike 1
6.25	etc. with spike 2
12.50	etc. with spike 3
18.75	etc. with spike 4

2. Using a calculator with linear regression curve fitting ability, and with Y-X calculation capability, enter the X, Y pairs of data as in the above table.
3. Calculate the r^2 , slope, and intercept for the data.
4. To calculate specimen values (controls or unknowns), enter the blank-corrected A (Y) values into the calculator, and calculate X (ng/mL).

NOTE: These two approaches will give essentially equivalent results if:

- 1) the r^2 value for the regression equation is high (0.98 or higher), and
- 2) the calculated intercept for the equation is near zero (0-0.05).

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two-way analysis of variance, ANOVA, with the construction of quality control charts based on 95% and 99% confidence limits of the mean of duplicate measurements, as well as range charts (4).

Precision and accuracy of the analytical system will be monitored as follows:

- 1) Ten analytical runs will be performed to characterize all control materials used, with duplicate measurements performed per run.
- 2) Analysis of variance calculations will be performed on these 20 data points, and quality control charts will be generated by computer for X and range R.
- 3) A minimum of two control materials will be incorporated into each analytical run of 20 unknown specimens, and data obtained for these controls will be evaluated with the X and R charts from 2).

Blind Quality Control

Two types of blind quality control specimens will be incorporated into the system:

- 1) blind duplicate specimens will be prepared and inserted at an interval determined by the supervisor, usually one blind duplicate per 20 unknown specimens.
- 2) blinded control or reference material samples will be inserted into each analytical run of 20 specimens, at the minimum rate of one blind control per 20 specimens.

In both cases, the blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Run Format

In all cases, the following format will be used for specimen determination:

<u>SAMPLE #</u>	<u>Sample ID</u>
1	Blank
2-5 (or greater)	Calibration Curve
6	Control I
7-27	Specimens
28	Control II
29-33	Calibration Curve
34	Blank

Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (4):

X chart

- 1) A single X value falls above the upper 99% limit or below the lower 99% limit.
- 2) Two successive X values fall either both above the upper 95% limit or both below the lower 95% limit.
- 3) Eight X values in succession fall either all above the center line or all below the center line.

R chart

- 1) A single R value falls above the upper 99% limit.
- 2) Two successive R values fall above the 95% upper limit.
- 3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

- 1) Check for errors in recording levels of control samples, and if none are found,
- 2) Check and calibrate instruments before performing further analyses on analytical samples,
- 3) Reanalyze patient samples performed during the out-of-control run.

METHOD PERFORMANCE

Limits of Detection

Limit of detection is in a practical sense determined by two factors: 1) the slope sensitivity of the method, i.e., the response (in the case of atomic absorption the absorbance or absorbance-second measurement) for a given concentration or amount of analyte, and 2) the random noise of the instrumental system used in measurement, especially that noise at the measured response for the blank for the determination (5).

According to the recommendations in reference (5), the limit of detection will be defined as that concentration of analyte corresponding to an absorbance or absorbance-second measurement equivalent to three times the standard deviation of this signal measured at an analyte concentration at a "low" level. In symbolic terms, this becomes:

$$c_L = \frac{3s_B}{m}$$

where c_L is the concentration calculated to be the limit of detection, s_B is the standard deviation of the measurement of a blank or low concentration sample; and m is the slope of the calibration curve (change in absorbance or absorbance-seconds/change in concentration or amount of analyte). For a set of measurements at the 0.6-ng/mL level, the standard deviation was $2.08 \times 10^{-3}A$, which yields a detection limit of 0.28 ng/mL ($N = 6$).

Accuracy and Precision

At the present moment, there is only one commercial source of blood with certified target values (6). Since this material is not available to the laboratory, evaluation of precision and accuracy was performed by the determination of cadmium in a bovine blood pool spiked with cadmium. Both the unspiked or "base" material and the spiked material were analyzed for cadmium with the described procedure. Precision at the "medical decision" level of approximately 5 ng/mL was 10% CV (total CV, including within- and among-day components). Performance of the proposed method for EPA quality control water samples, prepared as dilute aqueous nitric acid control materials, has consistently given $\pm 10\%$ accuracy; similar results have been obtained for National Bureau of Standards' SRM 1643a, Trace Elements in Water.

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1. Atomic Spectroscopy, 4(3): 69-86 (1983).
2. Anal Chemistry, 84: 1515 (1982).
3. Fresenius Z. Anal Chem, 300: 372-80 (1980).
4. A Statistical Quality Control System for the Clinical Laboratory. In Selection and Implementation of Methods in Clinical Chemistry, Commission on Continuing Education, Council on Clinical Chemistry, American Society of Clinical Pathology, Chicago, Illinois (March 1975).
5. Winefordner J and Long G. Limit of detection--a closer look at the IUPAC definition," Anal Chem, 55 (7): 713A-719A (1983).
6. Behringwerke AG, Product Bulletin, Frankfurt, FRG, (June 1981).

GRAPHITE FURNACE PROCEDURE FOR URINARY ARSENIC

(Revised August 30, 1983)

INTRODUCTION

Determination of arsenic in urine is a valuable part of many epidemiological and environmental health surveys, since urine arsenic reflects body burden, particularly recent undue absorption. Since the estimated half life of arsenic in the body is comparatively short, it is imperative that acute exposure cases be "sampled" as soon as possible after exposure in order to accurately evaluate potential toxic risk.

This method is based in principle on the use of nickel as matrix modifier (1-3) in order to thermally stabilize arsenic in the graphite furnace, presumably by the formation of a stable nickel arsenide. Much of the awkwardness and tedium of manual hydride methods is avoided in this automated method. The Zeeman background correction system offers the increased correction capacity needed at the primary resonance line of arsenic, 193.7 nm, well into the "short" UV range where salts and other matrix residues absorb strongly, causing high background absorbances.

EQUIPMENT

AA Spectrophotometer, P-E Zeeman/5000 with AS-40 Autosampler;
Data Station 10
L'vov Platform, PE catalog # B-0109-324.

REAGENTS

Water: Ultrapure water, prepared by a Milli-Q polishing system is used throughout.

Triton X-100: Scintillation Grade (Eastman Kodak) is used to prepare 0.10% v/v solution by volumetric dilution with ultrapure water.

Matrix Modifier

Nickel nitrate, 1000 mg/L, made by dissolving 4.953 g
 $\text{Ni(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in 200 mL ultrapure water, diluting to 1 L with
ultrapure water.

Stock and Working Arsenic Solutions. Arsenic, 1000 mg/L is used as received from Fisher (Cat. #So-A-450) or J.T. Baker (Cat. #6919-1). A 10-mg/L intermediate stock is prepared weekly by volumetric dilution of 1.00 mL stock to 100 mL; spiking solutions are prepared daily at 0.25, 0.50, 1.00, and 1.50 mg/L arsenic, also by volumetric dilution.

SPECIMEN COLLECTION

The judicious adherence to this protocol ensures urine specimens and samples taken therefrom to be of high quality in terms of minimal contamination from the ambient dust of air/clothes/skin.

1. Instruct subject to wash genitalia and hands prior to voiding and to be either naked or to wear clean underclothes (NO OUTERCLOTHING) when voiding into the collection bottle. A complete body washing (shower) with soap and water is recommended.

2. Collect 100+ mL of a first-morning void urine specimen in a 130-mL collection container (B+D 133 mL containers Cat. #4013 have been satisfactory).
3. Mix specimen thoroughly and decant 50-mL volume into a 50-mL plastic container (Falcon Cat. #2098 has been satisfactory).
4. Add 500 uL of ultrapure (ultrapure grade or equivalent) nitric acid, and mix specimen thoroughly.
5. Refrigerate collected specimen, and ship refrigerated to laboratory.
6. Prepare a "blank" with 50 mL ultrapure water, acidified with 500 uL of the nitric acid used in acidifying urine specimens. Return to laboratory with acidified urine specimens as a "blank."

ANALYTICAL PROCEDURE

1. Pipet 1.00 mL urine specimen or control into a precleaned 4-mL plastic vial.
2. Add 1.0 mL 4% v/v ultrapure nitric acid, prepared from J.T. Baker "ULTREX" grade or GF Smith "Redistilled" grade nitric acid.
3. Add 0.20 mL 0.10% v/v Triton X-100 and mix resulting solution thoroughly by vortexing 10-20 sec.
4. For the matrix-matched standard addition procedure, add 10 uL of the four standards above to four precleaned autosampler cups.
5. Pipet 250 uL of diluted urine into four of the four cups, pipetting the remaining diluted urine into a fifth cup.
6. With the AS-40 set for 10-uL sample volume and 10-uL alternate (matrix modifier) volume, pipet 10 uL of diluted urine, followed by 10 uL of matrix modifier into the graphite furnace.
7. Measure the absorbances of the prepared solutions in triplicate.

CALCULATIONS

Since the slopes of the curves prepared from random urine have been shown to vary up to $\pm 100\%$, the method of additions is used for determination of the unknown specimen concentrations. In the format above, the standard additions correspond to addition of 22, 44, 88, and 132 ng/mL arsenic to the original urine specimen.

In order to calculate the unknown concentrations, the following Table is constructed:

Specimen	ng/mL as added	Average A	A(1.04)-dilution	A(1.04)- A_{unspiked}
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unspiked	0	A	A	0
+ 10 uL 0.25 PPM	22 ng/mL	A_1	$A_1(1.04)$	$A_1(1.04) - A$
etc.				

With the entries in the last column to the right $A(1.04) - A$, entered as "Y," and ng/mL added as "X," perform a linear regression analysis on the data. Calculate slope, intercept, and r-squared terms.

Typical values for these parameters are:

slope	0.40 - 0.60
intercept	0 - 0.10
r squared	0.98

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two-way analysis of variance, ANOVA, with the construction of quality control charts based on 95% and 99% confidence limits of the mean of duplicate measurements, as well as range charts (4).

Precision and accuracy of the analytical system will be monitored as follows:

- 1) Ten analytical runs will be performed to characterize all control materials used, with duplicate measurements performed per run.
- 2) Analysis of variance calculations will be performed on these 20 data points, and quality control charts will be generated by computer for X and range R.
- 3) A minimum of two control materials will be incorporated into each analytical run of 20 unknown specimens, and data obtained for these controls will be evaluated with the X and R charts from 2).

Blind Quality Control

Two types of blind quality control specimens will be incorporated into the system:

- 1) blind duplicate specimens will be prepared and inserted at an interval determined by the supervisor, usually one blind duplicate per 20 unknown specimens.
- 2) blinded control or reference material samples will be inserted into each analytical run of 20 specimens, at the minimum rate of one blind control per 20 specimens.

In both cases, the blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Run Format

In all cases, the following format will be used for specimen determination:

<u>SAMPLE #</u>	<u>Sample ID</u>
1	Blank
2-5 (or greater)	Calibration curve
6	Control I
7-27	Specimens
28	Control II
29-33	Calibration Curve
34	Blank

'Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (4):

X chart

- 1) A single X value falls above the upper 99% limit or below the lower 99% limit.
- 2) Two successive X values fall either both above the upper 95% limit or both below the lower 95% limit.
- 3) Eight X values in succession fall either all above the center line or all below the center line.

R chart

- 1) A single R value falls above the upper 99% limit.
- 2) Two successive R values fall above the 95% upper limit.
- 3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

- 1) Check for errors in recording levels of control samples, and if none are found,
- 2) Check and calibrate instruments before performing further analyses on analytical samples,
- 3) Reanalyze patient samples performed during the out-of-control run.

METHOD PERFORMANCE

Limits of Detection

Limit of detection is, in a practical sense, determined by two factors: 1) the slope sensitivity of the method, i.e., the response (in the case of atomic absorption, the absorbance or absorbance-second measurement) for a given concentration or amount of analyte, and 2) the random noise of the instrumental system used in measurement, especially that noise at the measured response for the blank for the determination (5).

According to the recommendations in reference (5), the limit of detection will be defined as that concentration of analyte corresponding to an absorbance or absorbance-second measurement equivalent to three times the standard deviation of this signal measured at an analyte concentration at a "low" level. In symbolic terms, this becomes:

$$c_L = \frac{3s_B}{m}$$

where c_L is the concentration calculated to be the limit of detection; s_B is the standard deviation of the measurement of a blank or low concentration

sample; and m is the slope of the calibration curve (change in absorbance or absorbance-seconds/change in concentration or amount of analyte). For a set of measurements at the 8.5-ng/mL level, the standard deviation was 1.96×10^{-3} A.sec, which yields a limit of detection of 4.0 ng/mL ($N=4$).

Accuracy and Precision

Accuracy and precision of the urinary arsenic method have been estimated from determination of two different urine materials: a) a pooled urine from an epidemiological study of arsenic in Fairbanks, Alaska, well water; and b) a commercially available urine material (Fisher Urichem Level II). Target value for the Alaska material was established with a published hydride evolution method (6); the Level II by referee laboratories. Precision at the "medical decision" level of approximately 100 ng/mL was 18% (total CV, including within and among-day components), with agreement of ± 15 ng/mL with hydride values (6).

References

1. Benard H and Pinta M, Atomic Spectroscopy, 3: 8-12 (1982).
2. Freeman H et al., Atomic Absorption Newsletter, 15: 49-50 (1976).
3. HGA Recommended Conditions for Arsenic, Version 1.0, Perkin-Elmer Corp., Norwalk, CT, 1982.
4. A statistical quality control system for the clinical laboratory. In Selection and Implementation of Methods in Clinical Chemistry, Commission on Continuing Education, Council on Clinical Chemistry, American Society of Clinical Pathology, Chicago, Illinois (March 1975).
5. Winefordner J and Long G. Limit of detection--a closer look at the IUPAC definition. Anal Chem, 55(7): 713A-719A (1983).
6. Cox DH, Jo Anal Tox, 4: 207-11 (1980).

INSTRUMENTAL PARAMETERS FOR DETERMINATION OF URINARY ARSENIC

Wavelength	193.7 nm
EDL Power	8W
Slit	0.7 LOW
Signal Mode	ABS
Data Station 10	ON-PRINT ON
Read Time	5 sec
Inert Gas	Argon
Inert Gas Flow	300 mL/min; 20 mL/min@
	ATOMIZE
Zeeman Background	
Corrector	ON
Furnace Type	Pyrolytic

Temperature Program

DRY	180 C 20sec Ramp/25 sec Hold
CHAR	1150 C 5sec Ramp/15 sec Hold
ATOMIZE	2400 C 0sec Ramp*/5 sec Hold

*Maximum Power Heating Used

GRAPHITE FURNACE PROCEDURE FOR URINARY LEAD/CADMIUM

(Revised August 26, 1983)

EQUIPMENT

AA Spectrophotometer-Perkin Elmer Model 372 with Model 500 furnace, Model AS-1 autosampler, and Model 56 Recorder or Perkin Elmer Zeeman/5000 with Model AS-40 autosampler, and Model 10 Data Station.
L'vov Platforms - Perkin Elmer catalog # B-0109-324.

REAGENTS

Water: Ultrapure water, prepared by a Milli-Q polishing system is used throughout.

Triton X-100: Scintillation Grade (Eastman Kodak) is used to prepare 0.10% v/v solution by volumetric dilution with ultrapure water.

Matrix Modifier:

- A. Cadmium - make up a solution 4% by volume ultrapure nitric acid (GF Smith redistilled or Baker ULTREX) and 0.001% by volume Triton X-100, prepared by diluting 4.0 mL nitric acid and 1.0 mL 0.10% by volume Triton to 100 mL with ultrapure water.

- B. Lead - make up a solution 4% by volume ultrapure nitric acid and 1% weight by volume ammonium phosphate.

NOTE: These solutions should be checked daily for analyte content, since they readily contaminate with airborne dust, dirt, and/or by handling.

Stock and Working Lead/Cadmium Standards: A 1,000-mg/L stock solution of lead nitrate is prepared from 1.5985 g of NBS SRM 928 lead nitrate, diluted to 1L with 1.0% v/v nitric acid. A 1,000-mg/L stock solution of cadmium chloride is prepared from 1.6309 g of anhydrous reagent grade cadmium chloride, diluted to 1L with ultrapure water. These solutions are prepared every 6 months. With an intermediate lead standard of 10 mg/L (10 ppm) and an intermediate cadmium standard of 1 mg/L (1 ppm), combined working standards are made fresh daily as follows:

- A. 0.100 ml of 10 ppm Pb
 0.100 ml of 1 ppm Cd

qs to 10.0 ml with ultrapure water

Final Conc. = 0.10 ppm Pb
 10 ppb Cd

- B. 0.25 ml of 10 ppm Pb
 0.25 ml of 1 ppm Cd

qs to 10.0 ml with ultrapure water

Final Conc. = 0.25 ppm Pb
 25 ppb Cd

- C. 0.50 ml of 10 ppm Pb
0.50 ml of 1 ppm Cd qs to 10.0 ml with ultrapure water

Final Conc. = 0.50 ppm Pb
50 ppb Cd

- D. 0.75 mL of 10 ppm Pb
0.75 mL of 1 ppm Cd qs to 10. mL with ultrapure water

Final Conc. = 0.75 ppm Pb
75 ppb Cd

SPECIMEN COLLECTION

The judicious adherence to this protocol ensures urine specimens and samples to be of high quality in terms of minimal contamination from the ambient dust of air/clothes/skin.

1. Instruct subject to wash genitalia and hands prior to voiding and to be either naked or to wear clean underclothes (NO OUTERCLOTHING) when voiding into the collection bottle. A complete body washing (shower) with soap and water is recommended.
2. Collect 100+ mL of a first-morning void urine specimen in a 130-mL collection container (B+D 133-mL containers Cat.#4013 have been satisfactory).
3. Mix specimen thoroughly and decant 50-mL volume into a 50-mL plastic container (Falcon Cat. #2098 has been satisfactory).
4. Add 500 uL of ultrapure (ultrax grade or equivalent) nitric acid, and mix specimen thoroughly.
5. Refrigerate collected specimen, and ship refrigerated to laboratory.
6. Prepare a "blank" with 50 mL ultrapure water, acidified with 500 uL of the nitric acid used in acidifying urine specimens. Return to laboratory with acidified urine specimens as a "blank."

OVERALL HELPFUL HINTS AND REMINDERS:

1. Subject must be clean.
 2. Handle all materials with clean hands or use plastic gloves.
 3. Do not leave caps off containers for any long periods of time and handle caps carefully to avoid contamination of cap's inside.
 4. Tighten caps on shipping bottles to prevent leakage.
 5. Do not make ink, etc., markings on containers. Use labels.
- PLEASE RETURN ALL CONTAINERS AND OTHER MATERIALS, SINCE THE INITIAL COST AND THE TIME EXPENDED FOR CLEANING PROHIBITS REPEATED REPLACEMENTS FOR FUTURE ENDEAVORS.

ANALYTICAL PROCEDURE

1. In a 4-ml precleaned plastic vial, pipet 1.0 ml urine into 1.0 ml matrix modifier.
2. Vortex 30 sec.
3. Into four precleaned plastic autosampler cups, pipet 10 uL of the A, B, C, and D standards, using a 10-uL Eppendorf micropipet with disposable tips.
4. Add 250 uL of diluted urine to each of the three cups, using a 250-uL Eppendorf.

5. Transfer the remaining diluted urine to a fifth autosampler cup.
6. Add 500 ul of each of the remaining urine samples to a corresponding number of precleaned cups.
7. Measure the absorbance of a 20-uL aliquot of solution in triplicate, using attached instrumental parameters.

CALCULATIONS

1. Average absorbance change.

In this method the differences in corrected absorbances between A, B (df), C (df), D (df), and E (df) are calculated.

$$df = \frac{V + v}{v} = \frac{(0.250 + 0.010)}{0.250} \text{ mL} = \frac{0.26}{0.25} = 1.04$$

where V = added volume and v = original volume.

Construct the following table:

<u>Specimen</u>	<u>Spike (ng)</u>	<u>ng/mL Added*</u>	<u>Average Absorbance A</u>	<u>Corrected Absorbance A (1.04)</u>	<u>Corrected Absorbance Minus Unspiked</u>
Unspiked	None	0 ng/mL	A	A	0
+ 10 uL 0.10ppm Pb	1 ng	8 ng/mL	A ₁ Pb	A ₁ (1.04)	N
or 10 ppb Cd	0.1 ng	0.8 ng/mL	A ₁ Cd	A ₁ (1.04)	N ₁
+ 10 uL 0.25 ppm Pb	2.5 ng	20 ng/mL	A ₂ Pb	A ₂ (1.04)	N ₂
or 25 ppb Cd	0.25 ng	2.0 ng/mL	A ₂ Cd	A ₂ (1.04)	N ₂
+ 10 mL 0.50 ppm Pb	5.0 ng	40 ng/mL	A ₃ Pb	A ₃ (1.04)	N ₃
or 50 ppb Cd	0.50 ng	4.0 ng/mL	A ₃ Cd	A ₃ (1.04)	N ₃
+10 mL 0.75 ppm Pb	7.5 ng	60 ng/mL	A ₄ Pb	A ₄ (1.04)	N ₄
or 75 ppb Cd	0.75 ng	6.0 ng/mL	A ₄ Cd	A ₄ (1.04)	N ₄

Calculate the following values:

$$\frac{N_2}{2.5}, \frac{N_3}{5.0}, \frac{N_4}{7.5}$$

and average these values with N₁. This average is then divided by a)

8ng/mL (for lead) or b) 0.8 ng/mL (for cadmium) and set equal to cf.

Calculate unknown concentrations by multiplying the average absorbance of each unknown (after subjecting the blank) times the cf value for the appropriate metal.

*To illustrate the calculation, assume a 10-uL lead spike of 0.10 ppm to a 250-uL volume of 1:1 diluted urine:

$$\text{ng lead added} = (0.010 \text{ mL}) (0.10 \text{ ug/mL}) (1000 \text{ ug/ng})$$

$$= (0.01) (0.1) (1000) = 1 \text{ ng}$$

$$\frac{\text{ng/mL lead in analyzed solution}}{0.250 \text{ mL}} = \frac{1 \text{ ng}}{0.250 \text{ mL}} = 4 \text{ ng/mL}$$

$$\text{ng/mL in original urine} = (4 \text{ ng/mL}) \cdot (\text{dilution factor}) = 8 \text{ ng/mL}$$

2. Linear Regression

Construct the following table:

X (ng/mL added)	Y (N_1-N_5) - from previous calculation
---	---
0	0
8.0 (Pb)	N_1
0.8 (Cd)	N_1
20.0 (Pb)	N_2
2.0 (Cd)	N_2
etc.	

Enter the X, Y pairs into a calculator and perform linear regression analysis on the data points. Calculate slope, intercept, and r^2 for these data.

Typical Values:

	<u>Pb</u>	<u>Cd</u>
Slope	2.5-3.0	50-60
Intercept	0-5	0-5
r^2	0.98	0.98

Using the Y - X function, calculate unknown concentrations from the average absorbance of unknowns (after subtracting blank).

QUALITY CONTROL SYSTEM

Quality Control Statistics

The statistical format used for evaluation of quality control will be that of two-way analysis of variance, ANOVA, with the construction of quality control charts based on 95% and 99% confidence limits of the mean of duplicate measurements, as well as range charts (1).

Precision and accuracy of the analytical system will be monitored as follows:

- 1) Ten analytical runs will be performed to characterize all control materials used, with duplicate measurements performed per run.
- 2) Analysis of variance calculations will be performed on these 20 data points, and quality control charts will be generated by computer for X and range R.
- 3) A minimum of two control materials will be incorporated into each analytical run of 20 unknown specimens, and data obtained for these controls will be evaluated with the X and R charts from 2).

Blind Quality Control

Two types of blind quality control specimens will be incorporated into the system:

- 1) blind duplicate specimens will be prepared, and inserted at an interval determined by the supervisor, usually one blind duplicate per 20 unknown specimens.
- 2) blinded control or reference material samples will be inserted into each analytical run of 20 specimens, at the minimum rate of one blind control per 20 specimens.

In both cases, the blinds should be identical in appearance to the specimens, with the same containers, specimen volumes, and labelling. If desired, blind quality control specimens can be evaluated with the same statistical methods used for the control materials.

Run Format

In all cases, the following format will be used for specimen determination:

<u>SAMPLE #</u>	<u>Sample ID</u>
1	Blank
2-5 (or greater)	Calibration curve
6	Control I
7-27	Specimens
28	Control II
29-33	Calibration Curve
34	Blank

Action Limits

The analytical system will be declared "out of control" if one or more of the following events occur (1):

X chart

- 1) A single X value falls above the upper 99% limit or below the lower 99% limit.
- 2) Two successive X values fall either both above the upper 95% limit or both below the lower 95% limit.
- 3) Eight X values in succession fall either all above the center line or all below the center line.

R chart

- 1) A single R value falls above the upper 99% limit.
- 2) Two successive R values fall above the 95% upper limit.
- 3) Eight R values in succession fall above the center line.

If the system should be declared out-of-control, the following remedial action should be taken:

- 1) Check for errors in recording levels of control samples, and if none are found,
- 2) Check and calibrate instruments before performing further analyses on analytical samples,
- 3) Reanalyze patient samples performed during the out-of-control run.

METHOD PERFORMANCE

Limits of Detection

Limit of detection is, in a practical, sense determined by two factors: 1) the slope sensitivity of the method, i.e., the response (in the case of atomic absorption the absorbance or absorbance-second measurement) for a given concentration or amount of analyte, and 2) the random noise of the instrumental system used in measurement, especially that noise at the measured response for the blank for the determination (2).

According to the recommendations in the reference (2), the limit of detection will be defined as that concentration of analyte corresponding to an absorbance or absorbance-second measurement equivalent to three times the standard deviation of this signal measured at an analyte concentration at a "low" level. In symbolic terms, this becomes:

$$c_L = \frac{3s_B}{m}$$

where c_L is the concentration calculated to be the limit of detection; s_B is the standard deviation of the measurement of a blank or low concentration sample; and m is the slope of the calibration curve (change in absorbance or absorbance-seconds/change in concentration or amount of analyte). For a set of urinary lead measurements at the 8.1 ng/mL level, the standard deviation of the measurement was $2.88 \times 10^{-3}A$, which yields a limit of detection of 2.9 ng/mL ($N=5$). For a set of urinary cadmium measurements at the 0.2 ng/mL level, the standard deviation was $1.89 \times 10^{-3}A$, which yields a limit of detection of 0.1 ng/mL.

Accuracy and Precision

Urinary Lead

Accuracy and precision of the urinary lead method have been estimated from the determination of a commercially available urinary material (Fisher Scientific Urichem Level II) with lead values established by referee laboratories. Precision at the "medical decision" level of approximately 100 ng/mL was 9% CV (total CV, including within- and among-day components); with agreement of ± 5 ng/mL with target values by reference laboratories.

Urinary Cadmium

The reference material, SRM 2670, which would be the most appropriate material with which to establish accuracy for urine cadmium determinations, is not currently available to this laboratory. In order to evaluate method precision, a series of urine pools were prepared from pooled "normal" urine, which were spiked with: a) high purity cadmium (as acetate) to increase cadmium concentration by approximately 5 ng/mL, or b) concentrates used to prepare EPA quality control water samples (EPA samples 476-1,2,3). Preliminary characterization runs for these materials were performed. Precision at the "medical decision" level of approximately 5 ng/mL was 10% CV (total CV, including within- and among-day components). Performance on both EPA and NBS reference water samples has consistently given agreement of \pm 10% of the certified target value in the concentration range 0-10 ng/mL.

References

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PARAMETERS FOR DETERMINATION OF LEAD AND CADMIUM IN URINE
BY GRAPHITE FURNACE ATOMIC ABSORPTION

	Pb	Cd
Wavelength	283 nm	228 nm
Lamp Current	8 ma	6 ma
EDL Power	9.5 - 10 W	5 W
Slit	0.70 (ALT)	0.70 (ALT)
Signal Mode	Peak Height	Peak Height
Recorder	ABS	ABS
Read Time	5.0 sec	5.0 sec
Inert Gas	Argon	Argon
Inert Gas Flow	300 mL/min; 20 mL/min @ ATOMIZE	300 mL/min; 20 mL/min @ ATOMIZE
Deuterium Background		
Corrector	ON	ON
Furnace Type	PYROLYTIC-L'vov	PYROLYTIC-L'vov
TEMPERATURE PROGRAM:		
DRY	150°C 35 sec, 5 sec RAMP	Same as Pb
CHAR	600°C 25 sec, 5 sec RAMP	450°C 25 sec, 5 sec RAMP
ATOMIZE	2000°C 5 sec, 1 sec RAMP	2000°C 5 sec 1 sec RAMP
COOL	20°C 10 sec, 1 sec RAMP	20°C 10 sec, 1 sec RAMP

URINE B₂-MICROGLOBULIN

1.0

Title Page:

B₂-Microglobulin - approved method name.
B₂ micro test - "popular" name.
B₂m - abbreviation
May 12, 1983

2.0 Method Principles/Description

B₂-micro test is a competitive radioimmunoassay. B₂M in the sample competes with a fixed amount of ¹²⁵I labelled B₂m for the binding sites of anti-B₂M antibodies covalently bound to Sephadex particles. The competitive capacity is then compared with that of B₂M standards of known concentrations.

3.0 Definitions

Human B₂M is a low molecular weight protein (11,800 daltons) consisting of a single polypeptide chain of 100 amino acids. The protein is present on the surface of nucleated cells as the constant subunit of the classical transplantation antigens. It is released into the body fluids as a result of cell turnover.

4.0 Scope and Application4.1 Clinical Significance

Normally, only trace amounts of B₂M are excreted in final urine. However, the excretion of B₂M is markedly increased after minor derangements of the functional integrity of the proximal tubuli, e.g., after exposure to heavy metals, anticancer drugs, aminoglycosides, and anti-inflammatory compounds. B₂M is also excreted in increased amounts in the urine of patients with chronic pyelonephritis but not those with bacterial cystitis. The serum level of B₂M reflects the glomerular filtration rate more accurately than that of serum creatinine. The production rate of B₂M is increased in patients with some nonrenal diseases, particularly those involving the immune systems. In malignant lymphoma and multiple myeloma, the serum B₂M level can be related to turnover cell load, prognosis, and disease activity. The serum B₂M level is correlated to disease activity also in some nonmalignant diseases, including rheumatoid arthritis and hepatitis.

4.2 Limitations/Interferences

4.3 Normal Ranges

Serum Geometric \bar{x} = 81 ug/L
 Upper normal limit = 250 ug/L

<u>Urine</u>	<u>Age</u>	<u>\bar{x}(ug/L)</u>	<u>Upper normal limit(ug/L)</u>
	20-39	1,565	2,000
	40-59	1,894	2,600
	60-79	1,999	3,100

4.4 Manpower Requirements

One person is required to process samples and complete a run.

4.5 Analytical Throughput

44 samples/day

5.0 Safety

5.1 Reagent Toxicity or Carcinogenicity

None

5.2 Radioactive Hazards

^{125}I

5.3 Microbiological Hazards

None

5.4 Mechanical Hazards

None

5.5 Protective Equipment

Latex gloves, plastic-backed absorbent paper for desk, lab coat, safety glasses.

5.6 Training

5.7 Personal Hygiene

Avoid direct contact with the radioactive material by wearing protective clothing. Hands should be washed thoroughly with soap after any contact with radioactivity.

5.8 Disposal of Wastes

Compliance with the CDC Office of Biosafety guidelines is required. The procedure is as follows:

- 5.8.1 Place all dry, compactible waste (glassware, plastics, etc.) in a 13- x 12- x 24-inch plastic bag. Secure top with tape or rubber band.
- 5.8.2 Place all dry, noncompactible waste (paper, rubber gloves, etc.) in a 13- x 12- x 24-inch plastic bag. Secure top with tape or rubber band.
- 5.8.3 Isotope stock vials must be placed in small plastic bags and secured.
- 5.8.4 All radioactive waste containers (bags, boxes, etc.) must contain a label stating nuclide present, approximate activity, and date.
- 5.8.5 All uncontaminated outer packaging from radiation materials must have the warning labels removed or crossed out before disposal in the regular trash.

6.0 Sample Preservation and Handling

6.1 Sample Collection

6.1.1 Serum Samples

Blood should be collected by venipuncture, allowed to clot, and the serum separated by centrifugation.

6.1.2 Urine Samples

The following procedure for urine collection is recommended: the patient should first void the urinary bladder, then drink a large glass of water and collect a urine sample within 1 hour thereafter.

6.2 Sample Storage and Shipping Requirements

Serum samples can be stored for 1 week at 2°-8°C or for at least 1 year at -20°C. Urine samples with a pH between 6 and 8 can be stored for 2 days at 2 -8°C or for at least 2 months at -20°C.

6.3 Sample Handling

Urine should always be collected at high diuresis and the pH adjusted with 1.0 M NaOH to between 6 and 8 before storage.

7.0 Apparatus and Equipment

7.1 Description

1 500-ml or 1,000-ml graduated cylinder

1 400-ml or 800-ml beaker

1 10-ml graduated pipette

1 2.0-ml repeating pipette

1 each 50- μ l, 20- μ l, and 1,000- μ l micropipettes, with disposable plastic tips

146 polystyrene centrifuge tubes with round bottoms, 12 x 75 mm.

Redistilled water, absorbent paper, plastic film, vortex mixer, magnetic stirrer, magnetic stirring bar, centrifuge (swingout bucket), horizontal shaker, and ^{125}I gamma counter.

7.2 Routine Calibration

7.2.1 Pipettes should be calibrated according to instructions supplied by the manufacturer.

7.2.2 Gamma counter should be calibrated by counting a certified ^{129}I instrument standard.

7.3 Maintenance

7.4 Trouble Shooting

<u>Problem</u>	<u>Possible Cause</u>	<u>Solution</u>
Low activity	Radioactive decay	Check expiration date of tracer.
	Counter problem	Check counter efficiency.
	Aliquot error	Check volume of tracer used in assay.
Low binding (B_0)	Improper incubation conditions	Refer to manufacturer's recommendations. Equilibrate reagents to room temperature.
	Inadequate pelleting	Check centrifugation (time, recommended g force, etc.)
	Problem with supernatant removal	Follow recommended decanting procedures.
	Insufficient antibody	Check volume used in assay. Check storage, expiration date.
	Aged tracer	Check expiration date of tracer.
Poor duplication	Inadequate pelleting	Check centrifugation (time, G force, etc.)
	Problem with supernatant removal	Follow recommended procedures for supernatant removal.
	Variable pipetting	Calibrate pipets and/or dispensers.
	Improper reagent use	Equilibrate reagents to room temperature and mix before use.
High nonspecific binding	Addition of primary antibody to blank (reads same as "0" binding)	Do not add primary antibody to blank.
	Inadequate removal of supernatant	Check for retained liquid in blank tubes.
	Tracer deterioration	Check "0" binding, expiration date of tracer and storage conditions.
Flat curve	Insufficient standard	Check standard addition.
	Excess antibody	Check antibody addition.

Curve shift to right	Insufficient standard	Check standard addition.
	Excess tracer	Check tracer addition.
	Improper incubation conditions	Refer to manufacturer's recommendations.
		Equilibrate reagents to room temperature.
	Excess antibody	Check antibody addition.
Curve shift to left	Excess standard	Check standard addition.
	Insufficient tracer	Check tracer addition.
	Insufficient antibody	Check antibody addition.
Low values	Insufficient aliquot	Check volume used in assay.
	Improper sample storage or use	Check proper storage conditions. Do not acidify samples.
	Erratic curve	Check typical standard curve.
High values	Excess sample aliquot	Check volume used in assay.
	Inadequate pelleting	Check centrifugation and decanting procedures.
	Erratic curve	Check typical standard curve.

8.0 Reagents and Standard

- 8.1 Chemicals and Standards
Phadebas^R B₂-micro Test Kit, Catalog number (Pharmacia Diagnostics, Uppsala, Sweden)
- 8.2 Procedures for Evaluating New Lots of Chemicals and Standards
Reagents from Phadebas^R B₂M test packages with different lot numbers should neither be pooled nor interchanged. Also, they are not interchangeable with reagents in other tests from Pharmacia Diagnostics. Overlapping analytical runs should be made to confirm identical response with new reagent lots.
- 8.3 Reagent Preparation
 - 8.3.1 Phadebas^R B₂M test buffer solution.
Add 5 mL (1 vial) Tween solution to 300 mL deionized water; thereafter dissolve 8.3 g (1 vial) buffer substance powder in this Tween-water solution.
 - 8.3.2 Prepare Sephadex^R - Anti-B₂M Complex by adding 7.0 mL deionized water. Allow the suspension to stand for 2 min. Mix and put a magnetic stirring bar into the vial.
 - 8.3.3 ¹²⁵I-B₂M Solution
Reconstitute the ¹²⁵I-B₂M by adding 5.5 mL deionized water. Allow the solution to stand for 1 min. before mixing.
- 8.4 Calibrator Preparation
Reconstitute the B₂M calibrators by adding 1,000 uL deionized water to each vial. Allow the solutions to stand for 1 min. before mixing. To prepare a 1-ug/L calibrator, make a 1:10 dilution of the 10-ug/L calibrator with buffer solution.

9.0 Daily Operating Procedures

Label and arrange the test tubes. Each determination should be performed in duplicate for both calibrators and unknowns. A calibration curve should be prepared on each test occasion. Avoid dispensing solution onto the walls of the tubes.

- 9.1 Pipette 50 uL of Phadebas^R B₂-micro buffer solution (= zero calibrator) into tubes 1-2.
- 9.2 Pipette 50 uL of the B₂-microglobulin calibrator solutions 1,10, 25, 75, 200, and 500 ug/L into tubes 3-14.
- 9.3 Pipette 50 uL of diluted Unknowns into tubes 15-16, etc.
- 9.4 Pipette 50 uL of the ¹²⁵I-B₂-microglobulin solution into all tubes, including tubes T₁ and T₂. Tubes T₁ and T₂ contain only ¹²⁵I-B₂-microglobulin and are used to determine the total activity added. They are stoppered immediately and set aside.
- 9.5 Pipette 50 uL of the Sephadex^R -Anti-B₂-microglobulin complex suspension into all tubes except T₁ and T₂. The Sephadex^R -Anti-B₂-microglobulin complex suspension must be stirred continuously on a magnetic stirrer while it is being dispensed.
- 9.6 Mix (by vortexing) until the reaction solution turns a homogeneous green. Cover the tubes with plastic film or aluminum foil and incubate them on a shaker for 90 min. at controlled room temperature.

9.7 Separation

9.7.1 Add 2.0 mL Phadebas^R B₂-micro buffer solution into the tubes.

9.7.2 Centrifuge the tubes at 2,000 X g for 10 min. Use a swingout bucket.

9.7.3 Decant the supernatant by gently turning the tubes upside down without interrupting the turning movement. Do not shake the tubes! When the tubes are upside down, place them on an absorbent paper for 5 sec.

9.8 Determine the bound radioactivity in the tubes and the total activity (T_1 and T_2), using a gamma counter. When a gamma counter with 50% efficiency is used, 1 min. counting time will be sufficient. The background is determined by using an empty test tube.

10.0 Quality Control

10.1 Quality Control Protocol

Normal and high B₂M quality control samples of urine or serum will be included in each run.

10.2 Performance Evaluation Samples

Blind report samples will be included on a random basis.

10.3 Blanks

An empty test tube will serve as a counting blank.

10.4 External Proficiency Testing Program

None is available.

10.5 Special Requirements

None

11.0 Data Handling

11.1 Calculations

11.1.1 Calculations are performed by SPLINE function program on LKB RACKGAMMA II.

11.1.2 SPLINE function fitting can only be done by using a computer. An unknown concentration value is found by exactly the same general principle as in linear interpolation and polygonal interpolation. The curve segment on which the unknown lies is found, and the concentration is then calculated by using this equation.

11.2 Data Reporting Format

To be determined.

12.0 Literature References

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- 12.16 Simonsson, B, Wibell, L, and Nilsson, K: B₂-microglobulin in chronic lymphocytic leukemia. *Scand. J. Haematol* (1980) 24:174-180.
- 12.17 Spati, B, Child, JA, Kerruish, SM, et al: Behaviour of serum B₂-microglobulin and acute phase reactant proteins in chronic lymphocytic leukemia. *Acta Haematol.* (1980) 64:79-86.
- 12.18 Manicourt, D., Brauman, H. and Orloff, S: Plasma and urinary levels of B₂-microglobulin in rheumatoid arthritis. *Ann. Rheum. Dis.* (1978) 37:328-332.
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13.1 Quality Control System

13.1.1 Definitions

13.1.2 Quality Control Statistics

QC statistics are computed by nested analysis of variance (ANOVA) from replicate analyses of each QC pool. For urine assays two QC samples will be included in each run. These samples were prepared from a pool of urine from healthy volunteers. Aliquots of the pool were dispensed into Wheaton vials and stored frozen for later use as low QC samples. Aliquots of the pool after enrichment with B₂M calibrator were dispensed and frozen for later use as high QC samples.

13.1.3 Quality Control Techniques

13.1.3.1 Run Format. Duplicate aliquots (within vial) of low and high QC samples are included in each analytical run when study samples are analyzed. When establishing QC limits for these samples, quadruplicate aliquots from each QC sample were analyzed in the same run. Twenty runs were performed to establish QC limits before study samples were analyzed.

13.1.3.2 Precision estimates for low and high urine QC pools

Coefficient of Variation

<u>Sample</u>	<u>MEAN</u>	<u>STANDARD DEVIATION</u>	<u>AMONG DAY(df)</u>	<u>WITHIN VIAL(df)</u>
Low Urine	52.4 ug/L	6.82 ug/L	27.1% (14)	13.0% (46)
High Urine	181.1 ug/L	61.53 ug/L	4.0% (11)	34.0% (24)

13.1.4 Control Charts and Display of Control Data. Levy-Jennings charts of B₂M concentration versus date are made for each QC sample.

13.1.5 Laboratory Action Codes

13.1.6 Action Limits

13.1.7 Outlier Rules

13.1.8 Corrective Action Rules

13.1.9 Blind Quality Control



aca™ I/II/III TEST METHODOLOGY

CREA
CREATININE

INTENDED USE:

The CREA pack is used in the Du Pont aca™ discrete clinical analyzer to quantitatively measure creatinine in serum or urine.

SUMMARY:

The creatinine (CREA) method employs a modification of the kinetic Jaffe reaction reported by Larsen.¹ This method has been reported to be less susceptible than conventional methods to interference from non-creatinine, Jaffe-positive compounds.¹

Split sample comparison between the CREA method and a conventional Jaffe procedure on the Autoanalyzer® gave a correlation coefficient of 0.999 with no statistically significant bias.² Compared to a previous CREA method (Jaffe end point with protein removal), the present method gave a correlation coefficient of 0.994.²

®Registered trademark, Technicon Corp., Tarrytown, N.Y.

PRINCIPLES OF PROCEDURE:

In the presence of a strong base such as NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 510 nm due to the formation of this chromophore during a 17.07-second measurement period is directly proportional to the creatinine concentration in the sample.

Creatinine + Picrate $\xrightarrow{\text{NaOH}}$ Red chromophore
(absorbs at 510 nm)

REAGENTS:

Compartment ^a	Form	Ingredient	Quantity ^b
#2, 3, & 4	Liquid	Picrate	0.11 mmol
#6	Liquid	NaOH (for pH adjustment) ^c	

a. Compartments are numbered 1-7, with compartment #7 located closest to pack fill position #2.

b. Nominal value at manufacture.

c. See PRECAUTIONS.

PRECAUTIONS:

COMPARTMENT #6 CONTAINS 75 μL OF 10 N NaOH; AVOID CONTACT; SKIN IRRITANT; RINSE CONTACTED AREA WITH WATER.

USED PACKS CONTAIN HUMAN BODY FLUIDS; HANDLE WITH APPROPRIATE CARE.

FOR *IN VITRO* DIAGNOSTIC USE

MIXING & DILUTION:

The aca™ analyzer automatically aspirates a 200 μL sample of body fluid from the sample cup and injects it into the pack, along with 4.800 mL of Purified Water. The sample cup must contain a sufficient quantity of body fluid to accommodate the 200 μL sample size plus the 120 μL "dead volume" of the cup. Precise filling of the cup by the operator is not required. The micro sample cup insert, with a total volume of 500 μL and a "dead volume" of 10 μL , may also be used.

STORAGE INSTRUCTIONS:

Store under refrigeration (2–8°C). Do not freeze. Do not expose packs to temperatures above 35°C. Do not expose packs to direct sunlight.

EXPIRATION:

Refer to EXPIRATION DATE on the tray label.

SPECIMEN COLLECTION:

Normal procedures for collecting and storing serum and urine may be used for samples to be analyzed by the CREA method.³

KNOWN INTERFERING SUBSTANCES⁴

• Serum Protein Influence

Serum protein levels exert a direct influence on the CREA assay. The following should be taken into

account when this method is standardized as recommended with protein-containing materials:

- Aqueous creatinine standards or urine specimens will give CREA results depressed by approximately 0.7 mg/dL³ and will be less precise than samples containing more than 3 g/dL protein.
- All urine specimens should be diluted with an albumin solution to give a final protein concentration of at least 3 g/dL. Du Pont Enzyme Diluent (PN 790035-901) may be used for this purpose.
- High concentration of endogenous bilirubin (>20 mg/dL) will give depressed CREA results (average depression 0.8 mg/dL).⁴
- Grossly hemolyzed (Hb > 100 mg/dL) or visibly lipemic specimens may cause falsely elevated CREA results.^{2,7}
- The following cephalosporin antibiotics at the indicated serum concentrations have been shown to have no measurable effect on CREA results:

Cephalosporin	Drug Added (mg/dL)
Cefoxitin	2.5
Cefamandole	25
Cefazolin	10
Cephalexin	100
Cephalexidine	5
Cephalexin	50
Cephapirin	100
Cephadrine	10

- The single wavelength measurement used in this method eliminates interferences from chromophores whose 510-nm absorbance is constant throughout the measurement period.
- Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

TEST STEPS

When running analytical test packs the operator need be concerned only with loading the sample and appropriate test packs into a properly prepared instrument. The ACA automatically advances the packs through the test steps and prints the result. For details of sample preparation and pack processing, refer to Operating Instructions Section of the Instrument Manual.

Preset Creatinine Test Conditions

- Sample Size: 200 μ L
- Diluent: Purified Water
- Test Temperature: 37.0 \pm 0.1°C
- Reaction Period (Initiation to measurement): 29 seconds
- Measurement Period: 17.07 seconds
- Wavelength: 510 nm
- Type of Measurement: Rate
- Decimal Point Location: 000.0 mg/dL

ACA™ I/II analyzer:

- Assigned Starting Point: 990.0
 - Scale Factor: 0.2000 (mg/dL)/count^d
- ### ACA™ III analyzer:
- Assigned Offset C_0 : -1.000 E1 (-1.0 \times 10¹)
 - Linear Term C_1 : 2.004 E-1 mg/dL^d

d. The preset scale factor (linear term) was calculated from an absorbance to concentration relationship (sensitivity) of 17.54 mA/min/(mg/dL). Due to small differences in filters and electronic components between instruments, the actual scale factor (linear term) may differ from that given above.

PROCEDURE:

TEST MATERIALS

Quantity	Item	Du Pont Cat. #
1	ACA™ CREA Analytical Test Pack	701989901
1	Sample System Packet	702694901
1	Micro Sample System Packet and Micro Sample System Holders	702785000
	Dylux® Photosensitive Printer Paper	700036000
	Purified Water	704209901
	Cell Wash Solution	701864901

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CALIBRATION

The general calibration procedure is described in the Instrument Manual.

The following information should be considered when calibrating the CREA channel:

- Range of Linearity: 0—20 mg/dL
- Reference Materials: Protein containing primary standards* or secondary calibrators containing protein.
- Suggested Calibration Levels: 20, 5, 1 mg/dL

• **Starting Point (Offset C₀) Adjustment:**

For $\alpha\text{CA}^{\text{TM}}$ I analyzer, use the channel #8 adjustable zero offset (ZO) for the last two digits of the starting point. If adjustment of the first two digits of the starting point is required, replace the photometer method switching board.

For $\alpha\text{CA}^{\text{TM}}$ II analyzer, use adjustable starting point for all four digits.

For $\alpha\text{CA}^{\text{TM}}$ III analyzer, enter offset C₀ into Method Memory.

• **Scale Factor (Linear Term C₁) Adjustment:**

May be required for different pack lots.

• **Count By ($\alpha\text{CA}^{\text{TM}}$ I/II analyzer):**

One (1)

• **Readout Units:**

The αCA prints out in 0.1 mg/dL increments.

- Protein exerts a direct influence on the CREA method. Standard solutions of creatinine containing protein may be prepared as follows:

• **Reagents**

1. Creatinine, C₁₂H₁₁N₃O₄, F. W. = 113.2, crystalline, dehydrated.
2. Purified Water.
3. Bovine albumin, crystalline.

• **Bovine Albumin Solution (7.0 g/dL)**

Dissolve 70.0 g of crystalline bovine albumin in about 900 mL of Purified Water in a 1-liter volumetric flask. Adjust the volume to 1 liter with Purified Water.

• **Creatinine Stock Solution (100 mg/dL)**

Dissolve 500.0 mg of creatinine in 450 mL of the bovine albumin solution in a 500-mL volumetric flask. Adjust the final volume to 500 mL with the bovine albumin solution.

• **Creatinine Standards**

Creatinine standard solutions may be prepared using aliquots from the stock solution and bovine albumin solution as the diluent. These solutions should be refrigerated at 2–8°C. Sodium azide (CAUTION: Dangerous material. Handle with care.) may be added at a level of 10 mg/dL to inhibit the growth of micro-organisms.

NOTE

If the $\alpha\text{CA}^{\text{TM}}$ analyzer is calibrated with aqueous creatinine standards, serum based controls and human, protein containing specimens will analyze 0.6–0.8 mg/dL too high at all levels.

QUALITY CONTROL

Two types of quality control procedures are recommended:

- **General Instrument Check.** Refer to the Filter Balance Procedure and the Absorbance Test Method described in the Instrument Manual. Refer also to the ABS Test Methodology literature.

- **Creatinine Method Check.** At least once daily run a CREA test pack on a solution of known creatinine concentration such as an assayed control or calibration standard other than that used to calibrate the CREA channel. For further details review the Quality Assurance Section of the Chemistry Manual. The result obtained should fall within acceptable limits defined by the day-to-day variability of the system as measured in the user's laboratory. (See SPECIFIC PERFORMANCE CHARACTERISTICS for guidance.) If the result falls outside the laboratory's acceptable limits, follow the procedure outlined in the Chemistry Troubleshooting Section of the Chemistry Manual.

A standard deviation for five consecutive packs greater than 0.15 mg/dL for a level of 1.0 mg/dL or greater than 0.68 mg/dL for a level of 20.0 mg/dL indicates a possible system malfunction.

RESULTS:

The $\alpha\text{CA}^{\text{TM}}$ analyzer automatically calculates and prints the CREA concentration in mg/dL using the general scheme #2 illustrated in the Calculation of Results Section of the Chemistry Manual.

Information specific to the CREA calculation is listed below:

$\alpha\text{CA}^{\text{TM}}$ I/II analyzer:

- **Count By:** One (1)
- **Scale Factor:** 0.2000 (mg/dL)/count⁴

$\alpha\text{CA}^{\text{TM}}$ III analyzer:

- **Linear Term:** 2.004 E-1 mg/dL⁴

LIMITATION OF PROCEDURE:

CREA readouts in excess of 20 mg/dL should be repeated after diluting the sample with suitable protein base diluent to produce a sample concentration within the range of linearity. The resulting readout must then be multiplied by the dilution factor to give the CREA concentration of the undiluted sample.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing a letter code or word immediately following the numerical value should be held for follow-up. Refer to the Instrument Manual.

REFERENCE INTERVAL (Normal Range):^{5,7}

Males: 0.8 — 1.3 mg/dL
Females: 0.6 — 1.0 mg/dL

Each laboratory should establish its own reference interval for CREA as performed on the analyzer.

f. Reference interval data from 200 apparently healthy individuals (71 male, 129 female) between the ages of 19 and 72.

SPECIFIC PERFORMANCE CHARACTERISTICS:^g

REPRODUCIBILITY (Precision)

Within-Run^h

MEAN	S. D.	C. V. (%)	N
1.35 mg/dL	0.05	3.7	20
20.60 mg/dL	0.12	0.6	20

Day-to-Dayⁱ

MEAN	S. D.	C. V. (%)	N
1.36 mg/dL	0.05	3.7	20
20.65 mg/dL	0.37	1.8	20

LINEARITY:

0—20 mg/dL

- g. All SPECIFIC PERFORMANCE CHARACTERISTICS tests were run after normal recommended equipment quality control checks were performed (see Instrument Manual).
- h. N test packs, in series with a sample cup containing lyophilized serum base material, were used.
- i. One test pack per day for N days. A fresh sample of lyophilized serum base material was used each day.

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Clinical Systems





aca™ M/M TEST METHODOLOGY

UP
URINARY PROTEIN

INTENDED USE:

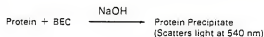
UP packs are used in the Du Pont aca™ discrete clinical analyzer to quantitatively measure protein in urine.

SUMMARY:

The UP method allows direct quantitation of protein in most urine samples within the normal and abnormal range. The UP method is an adaptation of the turbidimetric method of Iwata and Nishikaze.¹ Split-sample comparison between the UP method and biuret methods showed good correlation (see SPECIFIC PERFORMANCE CHARACTERISTICS).

PRINCIPLES OF PROCEDURE:

The UP method uses a two-pack, end-point technique to measure urinary protein. The UP-1 pack provides a sample blank at 540 nm. In the UP-2 pack, benzethonium chloride (BEC) precipitates urinary protein in an alkaline medium. Light scattering by the precipitate causes a decrease in light transmission. The decrease in light transmission is measured as absorbance at 540 nm. The absorbance difference between the UP-1 and UP-2 pack is related to the total protein concentration in the sample by means of a standard curve or mathematical function.



REAGENTS:

UP-1 (Blank)

Compartment ^a	Form	Ingredient	Quantity ^b
#1	Liquid	EDTA and Microbial Inhibitor	
#2 & #3	Liquid	NaOH ^c	
#4	Liquid	Surfactant and Microbial Inhibitor	

UP-2 (Reaction)

Compartment ^a	Form	Ingredient	Quantity ^b
#1	Liquid	EDTA and Microbial Inhibitor	
#2 & #3	Liquid	NaOH ^c	
#4	Liquid	Surfactant and Microbial Inhibitor	
#5 & #6	Liquid	Benzethonium Chloride ^c	45 μmol

a. Compartments are numbered 1-7, with compartment #7 located closest to pack fill position #2.

b. Nominal value at manufacture

c. See PRECAUTIONS.

PRECAUTIONS:

COMPARTMENTS #2 AND #3 IN UP-1 AND UP-2 PACKS EACH CONTAIN 65 μL OF 14 MOL/L NaOH: AVOID CONTACT; SKIN IRRITANT; RINSE CONTACTED AREA WITH WATER.

COMPARTMENTS #5 AND #6 IN UP-2 PACKS EACH CONTAIN 50 μL OF 0.45 MOL/L BENZETHONIUM CHLORIDE: AVOID CONTACT; SKIN IRRITANT; CORROSIVE TO MUCOUS MEMBRANES; RINSE CONTACTED AREA WITH WATER.

USED PACKS CONTAIN HUMAN BODY FLUIDS; HANDLE WITH APPROPRIATE CARE.

FOR *IN VITRO* DIAGNOSTIC USE

USE LIMITATIONS:

UP-1 AND UP-2 TEST PACK LOTS WHICH ARE SHIPPED TOGETHER MUST BE USED TOGETHER.

MIXING AND DILUTION:

The UP test is performed with two test packs which must be placed behind a sample cup in the order UP-1, UP-2. The aca™ discrete clinical analyzer automatically aspirates a 400 μL sample of urine and injects it into each test pack along with 4.600 mL of Purified Water. The sample cup must contain a sufficient quantity of sample to accommodate the total 800 μL sample size plus the 120 μL "dead volume" of the cup. Precise filling of the cup by the operator is not required.

STORAGE INSTRUCTIONS:

Store under refrigeration (2-8°C). Do not freeze. Do not expose packs to temperatures above 35°C or to direct sunlight.

EXPIRATION:

Refer to EXPIRATION DATE on the tray label.

SPECIMEN COLLECTION:

Normal procedures for collecting urine may be used. Samples to be analyzed by the UP method. Specimens stored at 4°C with no additives are stable for at least three days². Specimens stored under toluene or those containing sodium hydroxide (5%) or boric acid (100 mg/mL) are

acceptable. Specimens stored at room temperature with no additives showed an increase ($\approx 10\%$) in the protein level over a three-day period.

KNOWN INTERFERING SUBSTANCES:

- Magnesium at levels greater than 20 mg/dL will depress urinary protein results. Patients on intravenous MgSO₄ therapy may attain urinary magnesium levels high enough to depress results by as much as 50%.²
- Hydrochloric acid (0.1N) or boric acid (≥ 200 mg/mL) cause destruction of protein and should be avoided as additives at these concentrations².
- Bilirubin at a level of 1.9 mg/dL [32.5 μ mol/L] did not interfere². Bilirubin added to a level of 20 mg/dL [342 μ mol/L] increased the apparent protein level by 76 mg/L at a level of 200 mg/L and by 128 mg/L at a level of 1300 mg/L.
- The following substances at the levels shown had no effect on the UP method:

Ammonia,	180 μ g/dL [100 mmol/L]
Ascorbic acid,	100 mg/dL [5.7 mmol/L]
Creatinine,	20 mg/dL [1.8 mmol/L]
Glucose,	2.5 g/dL [138 mmol/L]
Phosphorus,	1 g/L [32 mmol/L]
Urea,	1.7 g/dL [600 mmol/L]
Uric acid,	50 mg/dL [3 mmol/L]
- Hemoglobin interference (see ANALYTICAL SPECIFICITY).

PROCEDURE:

TEST MATERIALS:

Quantity	Item	Du Pont Cat. #
1 pair	UP-1, UP-2 test packs and Graph Paper: GR DA [®]	705213901
1	Sample System Packet Dylux [®] Photosensitive Printer Paper Purified Water Cell Wash Solution	701989901 700036000 704209901 701864901

[®] Registered trademark. E. I. du Pont de Nemours & Co., Inc., Wilmington, DE.

d. Graph paper is packaged in each carton of UP-2 test packs. Each sheet of graph paper and each carton label has the letter code "GR DA" followed by a single digit number. For proper calibration, the letter/number code on the graph paper must match that on the carton label.

TEST STEPS:

When running test packs, the operator need be concerned only with loading the sample and appropriate test pack(s) into a properly prepared instrument. The α Ca™ discrete clinical analyzer automatically advances the pack(s) through the test steps. The α Ca™ II analyzer prints the result in milliabsorbance (mA) units which must be converted by the operator to concentration units using a previously prepared calibration curve or a mathematical function. The α Ca™ III analyzer prints the result in concentration units.

The actual mechanical travel of the test pack through the instrument is described in detail in Section III of the Instrument Manual for the α Ca™ analyzer.

Preset Urinary Protein Test Conditions

- Sample Size: 800 μ L (400 μ L/pack)
- Diluent: Purified Water
- Test Temperature: 37.0 \pm 0.1°C
- Reaction Period (initiation to measurement): 46 seconds
- Wavelength: 540 nm
- Type of Measurement: Two pack, end point
- Decimal Point Location: 0000.

α Ca™ II analyzer

- Assigned Starting Point^a: 9999. mA
- Scale Factor: 0.1000 mA/count
- Count By: One (1)

α Ca™ III analyzer

- Assigned Offset C₀: C₀, C₁, C₂, C₃ are matched to each lot of UP test packs.
- Assigned Linear Term C₁: See heading of graph paper packaged in cartons.
- Assigned Logit-Log Function Terms, C₂ and C₃:

e. For 2-pack methods on α Ca™ II analyzers, the starting point must be set below zero. Only a below zero starting point triggers the error circuitry which differentiates starting point printouts from result printouts on the patient report slip.

CALIBRATION:

The general calibration procedure is described in the Calibration/Verification chapter of the Instrument Manuals for the α Ca™ II and α Ca™ III discrete clinical analyzers. Calibration procedures should be followed for each new lot of test packs and must be repeated every three months for any one lot of test packs.

The UP channel on the α Ca™ analyzer should be calibrated over the assay range using five calibrators analyzed in duplicate.

- Assay Range: 60-2400 mg/L
- Suggested Calibration Levels: 90, 500, 1100, 1650, 2250 mg/L

- **Reference Material:** Primary standards or secondary calibrators such as Du Pont α Ca™ Urinary Protein Calibrator (P/N 790559901)

To calibrate the α Ca™ II analyzer for the UP method, construct a calibration curve on the graph paper provided according to the instructions in the Calibration/Verification chapter, Immunoassay paragraph of the Instrument Manual for the α Ca™ II analyzer. Adjustment of the starting point and scale factor is not required for calibration of α Ca™ II analyzers.

To calibrate the α Ca™ III analyzer for the UP method, follow the instructions in the Calibration/Verification chapter, Immunoassay paragraph of the Instrument Manual for the α Ca™ III analyzer. The theoretical constants for the logit-log function are matched to each UP pack lot and are given on the top of the graph paper.^d Adjustment of the OFFSET, (C₁) and LINEAR TERM, (C₂) may be required to calibrate the instrument.

If Du Pont α Ca™ urinary protein calibrators are being used, prepare them according to the instructions in the calibrator insert sheet.

QUALITY CONTROL:

Two types of quality control procedures are recommended:

- **General Instrument Check.** Refer to the Filter Balance Procedure and the Absorbance Test Method described in the Instrument Manual. Refer also to the ABS Test Methodology literature.
- **Urinary Protein Method Check.** At least once daily run a UP test on a solution of known protein concentration such as an assayed control or calibration standard other than that used to calibrate the UP channel. For further details review the Quality Assurance Section of the Chemistry Manual. The result obtained should fall within acceptable limits defined by the day-to-day variability of the system as measured in the user's laboratory. (See SPECIFIC PERFORMANCE CHARACTERISTICS for guidance.) If the result falls outside the laboratory's acceptable limits, follow the procedure outlined in the Chemical Troubleshooting Section of the Chemistry manual.

A possible system malfunction is indicated when analysis of a sample with five consecutive tests gives a coefficient of variation greater than 6.8% when calculated from mg/L results (or 8.8% when calculated from mA results) at a level of 340 mg/L, or gives a coefficient of variation greater than 8.7% when calculated from mg/L results (or 5.7% when calculated from mA results) at a level of 1400 mg/L. Refer to the procedure outlined in the Troubleshooting Section of the Chemistry Manual.

RESULTS:

For each UP test on the α Ca™ II analyzer, two results will be listed on the printout in the order UP-1 result, UP-2 result and both will be preceded by the letters GR DA. The

first result is the preset starting point. The second result is the sample measurement in milliabsorbance (mA) units. The urinary protein concentration in mg/L must be determined from the calibration curve constructed according to instructions in the Calibration/Verification chapter, Immunoassay paragraph of the Instrument Manual.¹

The α Ca™ III analyzer automatically calculates and prints the concentration of urinary protein in mg/L using the logit-log function described in the Field Evaluation Report.² Only one result is printed for each pair of UP packs.

- The calibration curve must be constructed on the correct graph paper. The OPERATOR MUST VERIFY THAT THE CORRECT GRAPH PAPER IS BEING USED. Each sheet of graph paper and each carton label has the letter code "GR DA" followed by a single digit number. The letter/number code on the graph paper must match that on the carton label and the entry for "DATE PLOTTED" must be within the previous 3 months.

LIMITATION OF PROCEDURE:

Readouts in excess of 2400 mg/L should be repeated after diluting the sample with Du Pont Purified Water to produce a sample concentration within the assay range. The resulting readout must then be multiplied by the appropriate dilution factor to give the UP concentration of the undiluted sample.

Results less than 60 mg/L should be reported as "less than 60 mg/L" instead of the numerical value. On α Ca™ III analyzers the Linear Limit error code "LL" will follow results less than 60 mg/L. For α Ca™ II analyzers, 60 mg/L is the lowest value shown on the graph paper.

α Ca™ II Analyzers:

The reporting system contains error messages to warn the operator of specific malfunctions. It is characteristic that the first printout of a 2-pack method will be the starting point followed by the code A. The second printout is the measured value for the sample in milliabsorbance (mA) units.

When one pack of a 2-pack method is decoded in the photometer, the computer is automatically programmed to receive pack 2 of that method as the next pack. However, circumstances where the next pack may not be the second pack of that test pair are possible, e.g.,

1. A single pack of a 2-pack method was used to check the starting point during calibration.
2. One pack of a 2-pack method fell off the transport pin.
3. Only one pack of a 2-pack method was loaded into the input tray.

In these cases, if the next test processed is also a 2-pack method, **ERRONEOUS RESULTS WILL BE PRODUCED ON BOTH TESTS.** This will be quite evident because the starting point and A error code will be printed for pack 2 of the second 2-pack method.

Examples		Printer
Pack Sequence		
Cases 1 & 3.	SAL-1	SAL - 999.9A
	UP-1	GR DA 5186 (will vary with sample)
	UP-2	GR DA 9999A

Case 2.	Pack Sequence	Printout
	SAL-1	SAL 999 8A
	SAL-2 ⁹	CDGDC 0 0 P
	UP-1	GR DA 9999A
	UP-2	GR DA 9999A

- g. SAL-2 was removed from the transport chain before it reached the photometer.

To return the computer to proper sequence:

1. Toggle the MOTOR HOLD RESET switch on the read-out board, or
2. Decode a header in the photometer from any 2-pack method, or
3. If the next test processed is a single pack method, the computer automatically resets and prints out the proper answer.

NOTE

These example printouts apply to α CA™ II analyzers only. If cases 1 or 2 occur on α CA™ III analyzers, the error message "SECOND PACK MISSED" is displayed and the filling station shuts down. To reset the filling station, remove the UP-1 pack from under the decode head in the filling station and push the ERROR OVERRIDE button. The SAL-1 pack is processed to the photometer where the error message "MISSING PACK" is displayed and printed on the report slip. If Case 2 occurs on α CA™ III analyzers, the error message "MISSING PACK" is displayed and printed on the report slip.

Any report slip containing another code or word immediately following the numerical value should be held for follow-up. Refer to the Instrument Manual for more details.

The UP method should not be used on instruments with round log amplifiers. The location of the log amplifier is shown in the Instrument Manual (α CA™ III analyzers have only square log amplifiers). Round log amplifiers can be identified by their round shape and gold color.

REFERENCE INTERVAL:

- Less than or equal to 165 mg/day
- Less than or equal to 135 mg/L

Twenty-four-hour urine collections were obtained from 195 apparently healthy adult individuals. This population consisted of laboratory personnel and their families and was nearly equally distributed between male (48%) and female (52%). One hundred and fifty-five (155) individuals were from southwestern Pennsylvania and forty (40) were from northcentral Texas.

The reference interval was derived non-parametrically by determining the 95th percentile.

Each laboratory should establish its own reference interval for UP as performed on the α CA™ analyzer.

SPECIFIC PERFORMANCE CHARACTERISTICS:

ASSAY RANGE:
60-2400 mg/L

REPRODUCIBILITY:

Control Material	Mean (mg/L)	Standard Deviation (% CV)	
		Within-Day	Between-Day
Urine Pool			
Level I	126	2.1 (1.7)	4.0 (3.2)
Level II	2164	53 (2.5)	69 (3.2)
URI-CHEM [®]			
Level I	475	1.4 (0.3)	3.6 (0.8)
Level II	988	3.2 (0.3)	5.5 (0.6)

Uri-Chem Controls: Fisher Diagnostics, Orangeburg, NJ.

CORRELATION:

Comparative Method	Regression Statistics		
	Slope	Intercept	r
Gel filtration-buret ¹	0.98	-76	0.989
Phosphotungstic acid-buret ¹	0.97	-86	0.976
Trichloroacetic acid-buret ¹	0.94	48	0.990
Coomassie Blue	0.96	50	0.986
Sulfosalicylic acid-turbidimetric	1.04	110	0.968
Trichloroacetic acid-turbidimetric	1.54	-58	0.926

- h. All SPECIFIC PERFORMANCE CHARACTERISTICS tests were run after normal recommended equipment quality control checks were performed (see Instrument Manual).
- i. Specimens at each level were analyzed in duplicate twice a day for twenty days. The within-day and between-day standard deviations were calculated by the analysis of variance method.
- j. Model equation for regression statistics is: [α CA™ analyzer result] = Slope \times [comparative method result] - Intercept.

ANALYTICAL SPECIFICITY:

Recovery of various proteins by the UP method is shown below:

Protein	% Recovery
Albumin	96
Gamma globulin	77
Transferrin	103
Orosomucoid	64
B-lipoprotein	92
Tamm-Horsfall	232
Lysozyme	11
Hemoglobin	10

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section 4



quality assurance

4-1. General.

To assure accurate and precise results from the **aca**, each method must be properly set up in the user's lab and thereafter must be maintained in control. The set-up procedure, either calibration for non-enzyme methods or verification for enzyme methods, is accomplished when the **aca** is originally installed or when a method is first put into routine use. Thereafter, a daily quality control program should be used to indicate changes in the **aca** system that may significantly affect the results of patient samples. This section of the manual presents Du Pont's recommendations for these two aspects of Quality Assurance.

4-2. Calibration of Non-Enzyme Methods.

A non-enzyme method can be set up following the calibration procedure in Section 5 of the **aca** Instrument Instruction Manual using standards prepared as described in the Test Methodology literature in the second half of this manual. Each new pack lot received by the laboratory should be calibrated. Since calibration requires two or three packs at each level, the precision of a new pack lot should be examined during this procedure. If the precision is unacceptable, refer to Chart 5-10 in Section 5.

When new calibration values are entered in the **aca**, the calibration should be verified by running several packs on the standards used. The calibration may also require verification during troubleshooting or after the replacement of some instrument components, notably the photometer filters.

4-3. Verification of Enzyme Methods.

~~X~~ Du Pont does not recommend lot-to-lot slope calibration of **aca** enzyme channels because there are no enzyme standards and lot-to-lot slope variations are generally less than $\pm 5\%$ as measured in the user's laboratory. However, in some cases offset adjustments may be necessary.

Specific data will be found in the Calibration section of each Test Methodology literature. When pack lots of an enzyme test are changed, the channel should be verified using the procedure given in Section 5 of the **aca** Instrument Instruction Manual. The recommended materials and the expected precision and linearity will also be found in the Test Methodology literature.

Enzyme verification materials can be either commercial control products or serum pools. They should be stable and have bottle values assigned by the user. If a lyophilized control product is used, at least three vials should be pooled to minimize the effect of vial-to-vial variation.

Bottle values can be correctly assigned only after the verification materials have been assayed using at least five pack lots. Preliminary bottle values can be estimated by assaying the materials with the first pack lot; these values are used for the verification of pack lots received before the bottle values are correctly established. The slope of **aca** readout versus bottle value for the first pack lot is, of course, 1.00. When a second pack lot is received, it should be verified using the bottle values estimated with the first pack lot. As mentioned above, the slope variations from lot-to-lot should be less than $\pm 5\%$, i.e., less than a 10% total range; thus, the slope of the second pack lot must be within 10% of the slope of the first pack lot. Since the slope of the first pack lot is 1.00, the second could be as low as 0.90 or as high as 1.10. The third pack lot received should also be verified using the preliminary bottle values estimated with the first pack lot. The slope of the third pack lot must be both within 10% of that of the first pack lot and within 10% of that of the second.

For example, if the first slope is 1.00 and the second is 1.06, then the third must be at least 0.96 (within 10% of the highest slope, 1.06) and at most 1.10 (within 10% of the lowest slope, 1.00). Each of the subsequent pack lots must be within 10% of all the previous lots.



The preliminary bottle values can be corrected once the slopes of at least five pack lots have been determined. The preliminary value should be multiplied by the center of the range of observed slopes, i.e., the average of the highest and lowest slopes, to obtain the corrected bottle value. Future verifications using the new values should result in slopes between 0.95 and 1.05.

Table 4-1 provides a summary of the procedure for determining bottle values.

When a new lot of control product or a new serum pool is used, a new "corrected" bottle value may be assigned by a crossover study using 20 packs from one pack lot and the following equation:

$$\text{new bottle value} = \frac{10 \text{ pack mean for new material}}{10 \text{ pack mean for old material}} \times \text{old bottle value}$$

Instrument bias between *aca*'s must be considered if a laboratory chooses to use a commercial control material having a bottle value that was not assigned using their *aca*. Slope differences as great as 10% can be obtained when two instruments are directly compared. For example, if the same control product was assayed with one pack lot on two different, properly functioning *aca*'s, it is possible that the slope on one instrument could be 0.95 and on the other, 1.05. If that control product was assayed on the first instrument with several pack lots, the range of slopes might be 0.90 to 1.00; with the same pack lots the range of slopes on the second instrument would be 1.00 to 1.10.

4-4. Quality Control Program.

Du Pont recommends that a sample of known activity or concentration (control) be analyzed at least once daily on the *aca* after acceptable performance has been demonstrated by the general instrument checkout procedures, i.e., filter balance and an ABS check. A control analysis should be run for each method to be used during the day. The results of the control analysis should be immediately recorded and if the results are outside acceptable limits, the cause of the out-of-control situation should be found and corrected before any more patient data is reported.

The control sample should be a material other than that used to calibrate (verify if an enzyme method)

the *aca*. If the control and calibration samples were identical, this could result in undetected error. If lyophilized quality control samples are used, the reconstitution technique recommended by the manufacturer's insert sheet or accompanying literature should be carefully followed. A fresh vial of lyophilized control should be used each day.

Hydration times should be kept as constant as possible, since the activities of some constituents change as the hydrated sample ages.

If only one control is used, a concentration or activity at the clinical decision making level, usually the upper limit of the normal range, is recommended. If a second concentration of activity is desired, a level near the upper limit of the range of linearity stated in the *aca* Test Methodology literature is recommended.

4-5. PREPARING A QUALITY CONTROL CHART.

In the following procedure it is assumed that the method has been properly set up as described previously in this section. The suggested technique of data handling and presentation is only one of several that may be used.

The quality control sample should be treated as a patient's serum on the *aca*. The tests should be repeated for 20 to 30 days with the same lot of test packs and control product. The individual determinations are used to calculate the mean (\bar{x}) value for the control, and the day-to-day standard deviation (SD) is then calculated using the following equation:

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - 1}}$$

With some electronic calculators the following equivalent formula for SD is easier to use:

$$SD = \sqrt{\frac{\sum (x_i^2) - N \bar{x}^2}{N - 1}}$$

Where:

x_i = Individual determinations

\bar{x} = $\frac{\sum x}{N}$, the mean of the individual determinations

N = Number of determinations



Table 4-1. Bottle Value Determination.

Procedure	Example
1. Estimate bottle values for verification materials with the initial pack lot. The slope of this pack is, by definition, 1.00.	1. Estimated bottle values are $V_1 = 138$ and $V_2 = 26$ $m = 1.00$ (m = slope)
2. When a new pack lot arrives, assay the verification materials with the new packs. Plot aca printout (y axis) vs estimated values from step 1 (x axis).	2. The slope obtained is 1.06.
Slope Specifications	
minimum acceptable slope = highest previous $m - 0.10$	previous $m = 1.00$ min. slope = $1.00 - 0.10 = 0.90$
maximum acceptable slope = lowest previous $m + 0.10$	max. slope = $1.00 + 0.10 = 1.10$ 1.06 is acceptable because it is within the range of 0.90 to 1.10
3. When another pack lot arrives, repeat step 2.	3. The slope obtained is 0.98
Slope Specifications	
Same as in step 2.	highest previous $m = 1.06$ lowest previous $m = 1.00$ min. slope = $1.06 - 0.1 = 0.96$ max. slope = $1.00 + 0.1 = 1.10$ 0.98 is acceptable because it is within the range of 0.96 to 1.10
4. Repeat step 2 with each new pack lot, always plotting aca printout vs bottle values estimated in step 1. After at least five different pack lots have been used, proceed to step 5.	4. The slopes obtained are between the previous high slope 1.06 and the previous low slope 0.98 and are, therefore, acceptable.
5. Calculate the correct bottle values by multiplying the estimated values by the average of the highest acceptable slope and the lowest acceptable slope.	5. highest $m = 1.06$.01 lowest $m = 0.98$.03 $\frac{1.06 + 0.98}{2} = 1.02$ $138 \times 1.02 = 140.8$ $26 \times 1.02 = 26.5$
6. When new pack lots arrive, plot aca printout vs the correct bottle values. The slopes are acceptable if they fall within the range of 0.95 to 1.05	.05 influence -

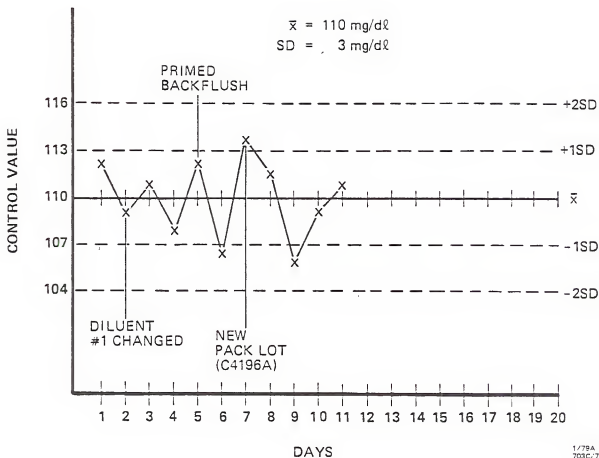


Figure 4-1. Example of Glucose Quality Control Chart.

A quality control chart of the type described by Barnett¹ and shown in figure 4-1 can then be prepared. Horizontal lines representing \bar{x} and action limits (frequently ± 2 SD) are drawn. The horizontal axis is graduated to represent days of the month. When a quality control test result falls farther away from the mean than the action limits, determination should be made whether the outlying point was caused by a systematic problem or was the result of normal statistical fluctuation. The troubleshooting charts in Section 5 will help. Each user should decide on action limits appropriate for a particular test. These could differ based on the level of confidence in control desired by the user. Typical action limits are ± 2 SD. Limits of ± 1 SD

might be appropriate where small systematic changes have a significant impact.

4-6. INITIATING THE PROGRAM.

When the laboratory initiates a quality control program with the **aca**, control must be assured during the initial 30-day period over which average values and typical SD are being determined. Tentative \bar{x} may be obtained by a one-time analysis of a five-vial pool of the control product. The average of a five-pack determination on this pool should give a reasonable estimate of the average (bottle) value to be expected. At this time the within-run precision should be assured as acceptable (consult the Test Methodology literature for typical performance characteristics). Tentative

¹ Barnett, R. M., M. D., "Clinical Laboratory Statistics," Little, Brown and Co., Boston, (1971), p. 77.



action limits may be obtained from any of the following criteria:

1. Past performances of an alternate methodology.
2. Tonk's Allowable Limits of Error² — Generally given as

$$\pm \frac{\frac{1}{4} \text{ normal range spread}}{\text{Mean of normal range}} \times 100$$
3. State of the art CV³
4. Specific performance characteristics given in the **aca** test methodology literature.

Typical performance characteristics are obtained from one laboratory with a given control product and test pack lot. Day-to-day precision in the user's laboratory should approximate that stated in the Test Methodology literature. If day-to-day precision established during the 30-day trial period is unacceptable or if one test result is outside ± 3 standard deviation, consult troubleshooting charts 5-1 or 5-2 in Section 5.

4-7. THE PROGRAM IN OPERATION.

The quality control chart shown in figure 4-1 is used to assess the performance of the method during any current month. Thus present performance is judged relative to past performance. Changes in the system that may affect daily performance are noted on the chart (the value of accurate record keeping is evident). At the end of the month, the \bar{x} and SD are recalculated to accommodate changes in the control lot or lot-to-

² Tonks, D., *Clin. Chem.*, 9, 217 (1963).

³ Barnett, R. M., *Amer. J. Clin. Path.*, 50, 671 (1968).

lot variability in the test packs. A cumulative SD (over several months) can be used as a more refined measure of day-to-day precision to be expected from the system.

If quality control results are normally distributed, 95% of the data points (19 out of 20) should fall within ± 2 SD of the mean. If more than one point within 10 determinations falls outside ± 2 SD limits, consult troubleshooting Charts 5-1 or 5-2 in Section 5. Note that 99.7% of the data points (299 out of 300) should fall within ± 3 SD of the mean. Thus, only one determination outside these limits indicates the need to immediately consult the troubleshooting charts.

Du Pont strongly recommends that the laboratory never completely run out of pack lots, buffers, or control products that have performed satisfactorily in the past, e.g., try not to run completely out of the pack lot presently in use before a new pack lot arrives and is found to be acceptable. New pack lots should be calibrated or verified when they arrive in a laboratory and when they can be directly compared to a satisfactory lot. For non-enzyme methods, the new C_1 and C_0 may be calculated and noted in the chemistry log sheets or printed out on Dylux paper. When the old lot is exhausted, the new C_1 and C_0 can be entered in the **aca**, and they can then be verified.

When a new quality control product or a new lot of control product is introduced, its performance should be evaluated in a 30-day trial while the system is known to be in control. The new \bar{x} and SD can be established and compared directly to the values of the old material during this trial.

ACA QUALITY CONTROL FORMAT

Quality control materials: Fisher Diagnostics Urichem Human Urine
(Lyophilized) Chemistry Control, Levels I and II, 25 mL/bottle.

<u>Name</u>	<u>Pack Code</u>	<u>Sample Size, uL</u>
1. Urinary Protein	(UP)	800 uL#
2. Urinary Creatinine	(CREA)	<u>200</u> uL##

Total Urine Required = 1,000 uL, or 1 mL

a 2-pack method, reflects 2- to 400-uL samples

urine is diluted 1:20 for analysis

- A. PRESTUDY PROTOCOL: To characterize instrument after calibration verified by manufacturer.

The protocol for urinary protein and creatinine is essentially the same as that for the serum analytes. Urinary protein, however, is a 2-pack method; pack #1 is a sample blank and pack #2 is the test pack. Only Level II of the Urichem controls has urinary protein; a 1:4 dilution with deionized water will provide a second level assessment in the normal range. Urinary creatinines must be diluted 1:20 with 3 g/dL albumin to be assayed on the ACA. (Calibration is based on serum creatinine and verified with urinary creatinine pools.)

Goal: Ten running days, 2 runs per day (AM and PM)

2 pools per run (Levels I and II for creatinine, Level II undiluted, and diluted 1:4 for urinary protein)
1 sample per pool, 2 packs per analyte

The Urichem controls are lyophilized in 25-mL quantities. One bottle of each level may be rehydrated with deionized water and dispensed in smaller aliquots, which are then frozen and used as required.

Level I: Rehydrate with 25 ml deionized water and mix well. Prepare 1:20 dilutions for CREA assay by diluting 50-uL aliquots of Level I with 950 uL of 3 g/dL human albumin. Mix well, freeze aliquots at -20°C.

Level II: Rehydrate with 25 ml deionized water. Prepare 10 1:20 dilutions for CREA assay as for Level I. Prepare 10 1,900-uL aliquots for UP (undiluted) assay. Dispense 10 500-uL aliquots and to each aliquot add 1,500 uL deionized water. Mix well. Freeze all aliquots at -20°C.

For each run, thaw one aliquot of each level or dilution. Mix contents of vials, fill cups, and arrange packs as follows:

Level 1 - 1:20 dilution

Cup 1 - 2 packs CREA = 400-uL sample

Level 2 - 1:20 dilution

Cup 1 - 2 packs CREA = 400-uL sample

Level 2 - 1:4 dilution

Cup 1 - 2 pack-sets UP = 1,600-uL sample

Level 2 - undiluted

Cup 1 - 2 pack-sets UP = 1,600-uL sample

Total packs consumed = 80 packs for creatinine and 80 pack-sets for urinary protein

Quality control materials required = 20 aliquots or dilutions per pool per analyte

B. WITHIN-STUDY PROTOCOL:

Pack lot variation will be minimized by having only one lot of packs per analyte, if possible, and one lot of each quality control pool.

1. Based on a maximum of 27 21-analyte profiles in 1 day, 4 sets of single packs for the normal level pool will be used:

- a. at beginning of day
- b. after 7-8 profiles (21 packs/profile)
- c. after 7-8 more profiles
- d. at end of day

1 vial of Level I will be prepared for each set.

2. CAP survey materials will be analyzed at least once during any study, possibly more often, if the surveys have been received during the course of a study.

Urine Chemistry - Survey U, Series 1

- CREA, UP - 2 packs or pack-sets per analyte per specimen

1. Specimen 1
2. Specimen 2

3. Blind duplicates

Blind duplicate samples will be collected in the field and presented in runs by the Special Activities group - two per day will be run. These samples may also consist of external control materials or pools for each analyte.

4. Replicate samples

Two replicates of samples scheduled for a day will be randomly repeated within that same day, if sample size permits.

C. BETWEEN-STUDIES PROTOCOL

Single packs will be analyzed for all analytes, using Levels I and II pools of Urichem.

Urichem, Level 1 - Cup 1 - 1:20 dilution, CREA

Level 2 - Cup 1 - 1:20 dilution, CREA

Cup 2 - 1:4 dilution, UP

Cup 3 - Undiluted, UP

If any analyte is out of control, analysis will be repeated to verify and check calibration.

HAIR ARSENIC/CADMIUM/LEAD

METHOD FOR SCREENING FOR SELECTED INORGANIC ELEMENTS IN ACID DIGESTS OF HUMAN HAIR BY INDUCTIVELY COUPLED ARGON PLASMA EMISSION SPECTROSCOPY

1.0.00 PRINCIPLE

Inorganic elements that may be found in human hair are derived from one or more of the following sources: (a) deposition during the synthesis of hair in the follicles; (b) external contamination of the hair from sweat; (c) external contamination from water and/or hair treatments (e.g., shampoos, hair conditioners, "permanent wave" solutions, tints and dyes); (d) external contamination from particulates or aerosols suspended in the atmosphere. It is hypothesized that a mild washing procedure will remove inorganic elements that are due to external deposition but will not damage the hair matrix to such an extent that the inorganic elements incorporated in the hair as it is growing are removed by the washing. The hypothesis is that such inorganic elements as are found in such a washed hair sample are in some way related to the chronic exposure or "body burden" of those elements.

Before analysis, the hair matrix is destroyed by digesting the sample in nitric acid and hydrogen peroxide. This frees the elements from the complex organic hair matrix. The elements in the acid digest are then identified by using inductively coupled argon plasma emission spectroscopy. The sample to be analyzed is aspirated into the plasma, where the extreme temperatures of the plasma excite the atoms. As these excited atoms return to a lower energy level, they emit energy at characteristic spectral wavelengths that may be detected by the sophisticated optical bench within the instrument. By comparing the intensity of emission in a sample with the intensity of known standards, the concentration of a particular element in a sample can be estimated.

2.0.00 SAMPLE COLLECTION AND PREPARATION

2.1.00 EQUIPMENT, REAGENTS, AND SUPPLIES

The following items are needed for hair collection:

- a) plastic zip-lock bags with labels affixed for storing the collected samples
- b) several pairs of high-quality stainless steel surgical scissors or plastic scissors for cutting the hair
- c) several plastic combs
- d) several aluminum or plastic hair clips
- e) 70% isopropyl alcohol (and appropriate containers) for cleaning scissors, clips, and combs

2.1.00 continued

- f) surgical gloves to fit the person(s) collecting samples
- g) information questionnaires to be filled out by those donating samples

The following are needed for hair washing, drying, weighing, and digestion:

- a) facilities with Class 100 air supply
- b) analytical balance capable of weighing accurately to a tenth of a milligram (0.0001 gram)
- c) aluminum digestion block shaped and drilled to hold Teflon digestion tubes
- d) hot plate to maintain the aluminum digestion block at 80-100°C
- e) 15-mL round-bottom Teflon tubes with screw-caps, calibrated for 10-mL volume
- f) Eppendorf pipets and pipet tips
- g) high-quality stainless steel surgical scissors or plastic scissors
- h) surgical gloves
- i) 15-mL conical-bottom plastic disposable capped centrifuge tubes (Falcon #2095)
- j) disposable 15- x 100 mm plastic petri dishes (Falcon #1010)
- k) miscellaneous Teflon bottles and beakers
- l) nitric acid, G. Frederick Smith, double-distilled from Vycor
- m) hydrogen peroxide, Mallinckrodt, 30%, analytical reagent grade
- n) sodium lauryl sulfate (1.0 g/100 mL) or ammonium lauryl sulfate (1.0 mL Agree shampoo/100 mL) in deionized water
- o) deionized water and Milli-Q deionized water

2.2.00 CLEANING OF LABORATORY SUPPLIES

The disposable plastic "labware" obtained from Falcon Plastics can usually be used as obtained. The Division of Environmental Hazards and Health Effects screens each new lot of such labware for inorganic contamination prior to use. All other plastics, glassware, and Teflon labware must be washed before being used.

The washing procedure involves two stages. The first stage is a detergent wash to remove much of the inorganic contamination as well as any organic residues. The second stage is an acid wash to remove the last traces of inorganic contamination.

The wash procedure is as follows. Labware to be cleaned is soaked for 3-24 hours in a 2% solution of Isoclean Decontamination Solution (Isolab, Inc.). After this detergent soak, the labware is rinsed thoroughly with deionized water and then soaked overnight (12-20 hours) in 30% hydrochloric or nitric acid (J.T. Baker analyzed reagent grade acid). After this acid soak, the labware is thoroughly rinsed (minimum of six rinses) with deionized water (Milli-Q reagent grade water) and placed in a clean work station providing Class 100 air for drying. The clean glassware is stored in the Class 100 work station or is packaged in plastic bags to prevent contamination before being used.

2.3.00 SAMPLE COLLECTION AND STORAGE

The individual collecting hair specimens should wear surgical gloves for his/her own personal hygienic protection and to avoid contaminating the hair samples with sweat from his/her hands. The gloved hands should be dipped in 70% isopropyl alcohol and dried between collections from different donors to prevent the transfer of scalp infection from one donor to the next. Likewise, combs, scissors, and hair clips should be soaked in 70% isopropyl alcohol between donors. The availability of several sets of combs, scissors, and hair clips facilitates the soaking, drying, and using cycles for these items.

Hair is cut from the occipital region of the scalp, between the top of the ears and the nape of the neck. Only the two inches of hair closest to the scalp, representing 2-6 months' recent growth, is used. The hair is cut so that the cuts show as little as possible.

Fasten hair that is not being cut out of the way with the aluminum (or plastic) hair clips. Cut 8-20 strands of hair from 10-20 different sites in the occipital region of the scalp with stainless steel surgical scissors (or plastic scissors, if available). Save only the two inches of hair growing next to the scalp. About 0.5 gram of hair is required for duplicate analyses of a hair sample, but a 1-gram sample is preferred to permit reanalysis of samples that have unusually high or low values for an element of interest.

2.3.00 continued

The collected hair is placed in a clean Zip-Lock plastic bag, and the donor's name and identification number are written on the label of the bag. The questionnaire completed by the donor (also showing the donor's name and identification number) is stapled to the sample bag.

When the samples and questionnaires are received in the laboratory, they are stored in a clean, dust-free environment at room temperature until they are prepared for analysis.

2.4.00 SAMPLE WASHING

The hair sample is transferred from the collection/storage bag to a 15- x 100-mm disposable plastic petri dish and covered with a 1.0% solution of either sodium lauryl sulfate or ammonium lauryl sulfate in deionized water (approximately 25 mL of detergent solution is required). The petri dishes are occasionally agitated (gently) to insure thorough contact of all the sample with the detergent solution. After 30 min, the detergent is poured off and the sample is rinsed (6 times) with deionized water (Milli-Q reagent grade). The samples are then left to dry in the uncovered petri dishes in a clean work station (Class 100 air).

2.5.00 SAMPLE WEIGHING

A 200-mg (approximate) portion of each washed, dried hair sample is accurately weighed (to the nearest tenth of a milligram) into a screw-cap Teflon digestion tube. Duplicate portions are taken from each sample. The tubes are capped after receiving the sample.

2.6.00 SAMPLE DIGESTION

The capped Teflon tubes containing the weighed hair sample are transferred to a digestion hood designed to provide a down-sweep of Class 100 air. A 1.0-mL aliquot of concentrated nitric acid (G.F. Smith double-distilled) is added to each tube with an Eppendorf pipet. The tubes are capped and the digestion is continued at room temperature until the hair is in solution (about 2 hours). The tubes are then transferred to an aluminum digestion block, placed in a digestion hood, and heated overnight (16-20 hours) at 80-110 °C.

After the acid digestion, the tubes are chilled in an ice bath and carefully uncapped (with gloved hands to avoid burns from acid vapors escaping under pressure). A 0.5-mL aliquot of 30% hydrogen peroxide is added to each tube with an Eppendorf pipet, and the tubes are capped and returned to the digestion block for 1.5-2.0 hours at 110°C. The tubes are then cooled to room temperature, and the volume of the samples is brought to the 10-mL calibration mark with deionized water (Milli-Q reagent grade). The samples are then poured into 15-mL disposable polystyrene centrifuge tubes (Falcon #2095) and capped tightly until analyzed.

2.6.01 NOTES ON SAMPLE DIGESTION

About 15 hair samples can be accurately weighed in duplicate and prepared for digestion in the early afternoon. The hair must have time to digest at room temperature before being heated to prevent a violent reaction. If this step is omitted, some samples react so vigorously that they boil out of the closed tubes at 80°C. By starting the digestion at room temperature and waiting until all the hair is in solution, we have never lost a sample because of boil-over.

The acid digest is yellow-green and clear (no particulate matter). The nitric acid oxidizes the protein, and the peroxide oxidizes the lipid material. This is not a complete digestion to carbon dioxide and water.

All manipulations of samples (except weighing) should be done in a hood or clean work station (Class 100 air) to minimize contamination.

At least four blank digestion tubes are prepared (containing no hair) for each digestion operation. A minimum of two samples of a solid hair quality control sample (described in a later section) are included in each digestion operation.

3.0.00 ANALYSIS BY ICAP EMISSION SPECTROSCOPY

Digested hair samples are analyzed by using a Jarrell-Ash Plasma AtomComp 1160 spectrometer connected to a Digital Equipment Corporation PDP 11/34 computer. The Jarrell-Ash fixed cross-flow nebulizer is connected to a Gilson Minipuls 2 peristaltic pump to assist in sample aspiration and nebulization. The principle of the method is presented in Section 1.0.00.

3.1.00 EQUIPMENT, REAGENTS, AND SUPPLIES

The following are needed for the analytical procedure:

- a) Jarrell-Ash Plasma AtomComp 1160 with PDP 11/34 computer
- b) Gilson Minipuls 2 peristaltic pump
- c) Argon, high purity
- d) J.T Baker Instra-Analyzed Atomic Spectral Standards (guaranteed 1000 ± 10 ppm from 99.99% pure metals or salts) for the following elements: Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sb, Sn, Se, Sr, Ti, V, Zn

3.1.00 continued

- e) Aldrich atomic absorption standard solutions (1,000 ppm) for the following elements: Au, Tl, P, Y
- f) National Bureau of Standards' Standard Reference Materials for the following: calcium carbonate (1.2488 g/500 mL); lithium carbonate (2.6624 g/500 mL); sodium chloride (1.2713 g/500 mL); potassium chloride (0.9535 g/500 mL)
- g) J.T. Baker Ultrex hydrochloric acid
- h) G. Frederick Smith nitric acid, double-distilled from Vycor
- i) J.T. Baker Ultrex acetic acid

3.2.00 CLEANING OF LABORATORY EQUIPMENT AND SUPPLIES

The procedure for the cleaning of laboratory supplies is the same as that described in Section 2.2.00.

The nebulizer/torch assembly from the ICAP is cleaned weekly under normal operating conditions used for hair analysis. If the instrument has been used for the analysis of trace elements in other matrices, then the cleaning protocol described for those matrices is used. The standard cleaning procedure for the nebulizer/torch assembly is as follows. The assembly is removed from the instrument, disassembled, and soaked in a warm solution (2%) of Isoclean Decontamination solution for 3-24 hours. This is followed by 6 rinses in Milli-Q reagent grade water and gently shaken to remove excess water. After the instrument has been assembled and installed, the argon used for the instrument is allowed to flow through the torch for several minutes to accomplish final drying. Too much moisture in the torch will prevent plasma from forming.

3.3.00 INSTRUMENT CALIBRATION AND SAMPLE ANALYSIS

The instrument is calibrated by using five mixtures of standards. These calibrator solutions are designated WAT1, WAT2, WAT3, WAT4, and WAT5. They are prepared from the stock standards (1,000 ppm) of each individual element. These solutions are prepared as follows:

WAT1: 15 mL G.F. Smith nitric acid diluted to 500 mL with Milli-Q reagent grade water. This is the blank calibrator solution.

WAT2: 15 mL G.F. Smith nitric acid and 5.0 mL of the individual stock standards (1000 ppm) for the following elements: Ca, Cd, Co, Cu, Mg, Mn, Pb, Zn. The volume is brought to 500 mL with Milli-Q reagent grade water.

3.3.00 continued

WAT3: 15 mL G.F. Smith nitric acid and 5.0 mL of the individual stock standards (1,000 ppm) for the following elements: Al, Ba, Be, Fe, Li, Mo, Na, Ni, Sb, Sn, Sr, Ti, Tl. The volume is brought to 500 mL with Milli-Q reagent grade water.

WAT4: 15 mL G.F. Smith nitric acid and 5.0 mL of the individual stock standards (1,000 ppm) for the following elements: As, B, Cr, P, Se. The volume is brought to 500 mL with Milli-Q reagent grade water.

WAT5: 15 mL G.F. Smith nitric acid and 5.0 mL of the individual stock standards (1,000 ppm) for the following elements: Au, V, Y. To this is added 50 mL of the potassium stock standard (1,000 ppm). The volume is brought to 500 mL with Milli-Q reagent grade water.

The calibrator solutions contain 10 ppm of each element except for potassium, which is at 100 ppm.

The calibrator solutions prepared as described above are used with ACT = CDGS (analytical control table CDGS), which uses the spectrum shifter for background correction. When ACT = CDC1 is used, 7.5 mL of Ultrex acetic acid is added to each calibrator solution before the final volume is brought to 500 mL. The acetic acid in these is added to correct for the nonspecific carbon interference in the digested hair samples. The spectrum shifter is not used in ACT = CDC1.

ACT = CDGS provides the following parameters:

- two burns per sample
- five seconds per burn
- spectrum shifter at 11

ACT = CDC1 provides the following parameters:

- three burns per sample
- ten seconds per burn
- spectrum shifter at zero
- acetic acid in calibrator solutions

The ICAP is calibrated in the intensity mode by using the appropriate ACT and the appropriate calibrator solutions. After being calibrated, the instrument is set to the concentration mode. The calibrator solutions are then read in the concentration mode as a check of the calibration. The pooled digested hair quality control sample (described in a later section) is analyzed as a check for between-day precision for each element.

3.3.00 continued

A standard solution containing 2 ppm of each element is analyzed periodically to monitor calibration. The system is washed between samples and standards to prevent cross-contamination. Analytical results for standards, blanks, digestion blanks, unknown samples, pooled hair digest quality control samples, and other quality control samples are printed out in the concentration mode as "ppm." Values recorded for duplicates are averaged to calculate a final value for a given sample.

3.4.00 QUALITY CONTROL

Since this method is considered an experimental method, still under development and evaluation, no formal quality control protocol has been established. At present, two quality control samples are used: a sample of hair obtained from one individual and a pool of excess hair digests. The pooled hair digest sample is used to monitor within-day and between-day precision. The solid hair sample has been rendered as homogeneous as we are able to make it, and it is used to monitor the overall reproducibility of the method, from weighing through analysis (the washing step is not included). As our experience and resources permit, additional quality control materials and procedures will be developed.

3.5.00 REPORTING OF ANALYTICAL RESULTS

This method is considered an experimental method, still under development and evaluation.

Table 1
ANALYTICAL GOALS SUMMARY

ANALYTE	QUALITY CONTROL MATERIALS	MATRIX	LINEARITY	ESTIMATED		
				PRECISION	DETECTION LIMIT	SAMPLE THROUGHPUT
Whole Blood Lead	1. Spiked Whole Blood 2. Internal Controls	Bovine Blood Bovine Blood	0 - 100 ug/dL	10% CV @ 15 ug/dL	2.0 ug/dL	40 samples/day
Erythrocyte Protoporphyrin	1. Standards (ProtoIX) 2. Internal Controls	Aqueous Human/Bovine	0 - 100 ug/dL	7% CV @ 28.5 ug/dL	1 ug/dL	100 samples/day
Whole Blood Cadmium	1. Spiked Whole Blood 2. Internal Controls	Human Blood Bovine Blood	0.5 -10 ng/mL	10% CV @ 6.9 ng/mL	0.25 ng/mL	40 samples/day
Urinary Arsenic	1. Spiked Urine 2. Internal Controls	Human Urine Commercial	0 - 250 ng/mL	5% CV @ 245 ng/mL	4.0 ng/mL	20 samples/day
Urinary Cadmium	1. Calibrators 2. Internal Controls	Human Urine Commercial	0 - 10 ng/mL	10% CV @ 4.6 ng/mL	0.1 ng/mL	20 samples/day
Urinary Lead	1. Calibrators 2. Internal Controls	Human Urine Commercial	0 - 120 ng/mL	9% CV @ 109 ng/mL	3.0 ng/mL	20 samples/day
Urine B ₂ -Microglobulin	1. Calibrators 2. Internal Controls	Human Urine Human Urine	1 - 500 ug/mL	14% CV @ 40.0 ug/L	1 ug/mL	40 samples/day
Urinary Creatinine	1. Calibrators 2. Internal Controls	Bovine Alb Soln Commercial	0 -20 mg/mL	4% CV @ 1.40 mg/dL	0.15 mg/dL	80 samples/hour
Urinary Protein	1. Calibrators 2. Internal Controls	Bovine Alb Soln Commercial	60 -2400 mg/L	3% CV @ 130 mg/L	60 mg/L	80 samples/hour
Hair Arsenic/Cadmium/Lead	Not Available	Under Development	Under Development			

METHOD MODIFICATIONS

All analytical methods were as outlined in the preceding sections of this appendix, with the following modifications.

URINARY LEAD - A 1.6% weight by volume solution of ammonium phosphate in 4% by volume nitric acid was used in place of a 1% solution of ammonium phosphate in nitric acid as a matrix modifier.

URINARY BETA-2-MICROGLOBULIN - 10 mg of sodium carbonate was added to each 10 ml of urine specimen. This replaced the use of 1.0 M NaOH to adjust the pH to between 6 and 8 before storage.

HAIR CADMIUM AND LEAD - Because of the limited sample size, the amount of sample weighed and digested was reduced by 50%, with a corresponding reduction in the volumes of the digestion reagents (nitric acid, hydrogen peroxide, and deionized water). The resulting decrease in the volume of digest necessitated a change in the analytical sampling scheme (a 2-burn sequence using ACT CDC 1 replaced the normal 3-burn sequence). Samples were washed in either a 1% SDS solution or in 95% ethanol (statistical analysis has shown that there is no significant difference between these two methods of washing in our laboratory).

The concentrations of several elements (e.g., Fe, Cr, Cu, Ni) in the SDS-wash of sample 312-1052 suggest a possible "steel" contamination. Although the Pb and Cd results do not seem to be affected, contamination may have occurred during the collection, preparation, and/or analysis of this specimen.

Lead values below 5.0 ug/g of hair are less than five times the detection limit of the method and are reported for comparative purposes only. All cadmium values are above five times the detection limit for cadmium.

HAIR ARSENIC - The modifications of the washing and digest method were the same as above for lead and cadmium. No ICAP values for arsenic are reported because all were below the detection limit of the method. The acid digests of the SDS-washed hair specimens were analyzed by using graphite furnace atomic absorption with L'vov platform and matrix modifier. The conditions chosen were similar to those described by W. Slavin et al., Atomic Spectroscopy, 4: 69-86, 1983, and F.J. Fernandez and R. Giddings, Atomic Spectroscopy, 3: 61-65, 1982, for arsenic in biological materials. Calibration was done by adding inorganic arsenic standards to a pooled hair digest. Instrumental conditions were:

DRY 200 C	5 sec	RAMP 30	sec	HOLD
CHAR 800 C	5 sec	RAMP 20	sec	HOLD
ATOMIZE 2400 C	1 sec	RAMP 5	sec	HOLD; 0 flow
COOL 20 C	1 sec	RAMP 10	sec	HOLD

An arsenic EDL was used as a resonance line source; conventional (deuterium) background correction was used. The applicability of conventional background correction was tested by absorbance measurements of the unspiked pool digest, with and without deuterium correction. The measured absorbance difference for this pool was about 0.03 AU, well within the correction capability of this system (maximum about 0.20 AU). The specimen digest were diluted 1:1 with a matrix modifier (1,000 ppm in nickel--as nitrate--and 4% v/v in nitric acid). The method of average slopes was used to calculate results, with specimens bracketed between two hair digest standard addition curves to compensate for any sensitivity change or instrument drift with time.

QUALITY CONTROL DATA FOR
ANALYTICAL RUNS

Twenty-five blood specimens collected from children in East Helena, Montana were analyzed for blood lead. The specimens were processed in two analytical runs. Values reported were means of duplicate determinations. One aliquot of each of three bench control pools was included in each run. Additional aliquots of one of these pools were included as blind controls, four in the first run and two in the second run. All control means were within the 95% limits shown below except the mean for the 83-33 pool in the second run, which was within the 99% limits. Units below for blood lead are ug/dl.

QC Mean Limits

Pool	UCL99	UCL95	Mean	LCL95	LCL99
83-33	7.03	6.51	4.87	3.23	2.71
DE8A03	17.09	16.15	13.19	10.22	9.28
DE8C01	37.74	36.53	32.70	28.88	27.67
blind	7.03	6.51	4.87	3.23	2.71

Fifty-two blood specimens were analyzed for cadmium in six analytical runs. All values reported were means of duplicate determinations. Included in each run were two aliquots of one bench control pool and one aliquot of another bench control pool. All means were within the 95% limits given below (blood cadmium units are ng/ml):

QC Mean Limits

Pool	UCL99	UCL95	Mean	LCL95	LCL99
High	8.95	8.58	7.43	6.28	5.92
PB83	0.797	0.674	0.286	-0.102	-0.225

Twenty-five blood specimens from children were analyzed for erythrocyte protoporphyrin (EP) in one analytical run. Four EP determinations were also made on each of four bench control pools. All means were within the 95% limits given below. Three of the ranges were within the 95% limits; the fourth was outside the 99% limits. With eight QC parameters, the probability of at least one being outside the 99% limits by chance is about 8%, which is larger than the 2% probability level used within the Division of Environmental Health Laboratory Sciences to warrant rejection of an analytical run. Six samples from an uncharacterized blind control pool were also included in the analytical run. These had a CV of 6.86%. Units for EP in the table below are ug/dl.

QC Mean Limits

Range of
Quadruplicate
Values Within Run

Pool	UCL99	UCL95	mean	LCL95	LCL99	UCL99	UCL95
2681	55.5	54.6	52.7	49.0	48.1	6.80	5.61
2781	129.6	126.7	117.7	108.7	105.8	17.46	14.41
2881	182.3	178.9	168.4	157.9	154.6	20.30	16.75
1882	38.7	37.9	35.5	33.1	32.4	5.27	4.34

Ninety urine specimens were analyzed for arsenic in 11 analytical runs. All values were means of duplicate determinations. One aliquot from each of two bench control pools included in each run. In addition, one to three aliquots of one of these pools were included in the first four runs as blind controls. All means were within the 95% control limits shown below, except the 476-1 pool mean in the fourth run, and one of the three blind control means (476-1 pool) for the third run both of which were within the 99% control limits. All units for urinary arsenic below are ng/ml.

QC Mean Limits

Pool	UCL99	UCL95	Mean	LCL95	LCL99
base	12.98	10.86	4.16	0	0
476-1*	38.79	35.26	24.13	12.99	9.47

*Bench and blind control

Seven pairs of blind split duplicates were also included in the analytical runs. The table below shows the results of measurements on the duplicate aliquots. Units for urinary arsenic are ng/ml.

Pair	Run Number(s)	Concentration 1	Concentration 2
1	7, 10	22.5	28.3
2	5, 3	4.0	16.1
3	3, 4	4.0	4.0
4	3, 3	4.0	4.0
5	1, 1	7.1	32.7
6	1, 2	4.0	4.0
7	2, 2	10.2	19.3

In four of these split duplicate pairs, the two values varied considerably. In each case, the value for the duplicate specimen (concentration 2) was higher than for the original specimen from which it was aliquoted (concentration 1). Thus, some contamination in the aliquoting process may account for the discrepancies. Similarly, values approaching the detection limit of the method (i.e., 4 ug/mL) are subject to a great deal of analytical variability, as reflected in the base pool control.

Forty urine specimens were analyzed for lead in two analytical runs. All values were means of duplicate determinations. One aliquot of each two bench control pools was placed at the beginning and at the end of each run. In the first run, one aliquot and, in the second run two aliquots of one of these pools were also included as blind controls. All means were within the 99% control limits given below, except the means for the base urine pool at the beginning of the first run and for the base pool at the beginning and end of the second run, which were within the 99% control limits. Units below for urinary lead are ng/ml.

QC Mean Limits

Pool	UCL99	UCL95	Mean	LCL95	LCL99
base	7.71	7.11	5.20	3.29	2.69
476-1*	42.42	39.33	29.58	19.83	16.74

*Bench and blind control

Three pairs of "blind" split duplicates were also included in the analytical runs. The table below shows the results of the determinations on the duplicate aliquots. Units for urinary lead are ng/ml.

Pair	Run Number(s)	Concentration 1	Concentration 2
1	1, 1	2.8	3.3
2	1, 2	2.8	2.8
3	2, 1	3.1	2.8

Fifty urine specimens were analyzed for cadmium in two analytical runs. All values were means of duplicate determinations. One aliquot from each of two bench control pools was included at the beginning and the end of each run. In addition, two aliquots from one of these pools were also included in each run as blind controls. All means were within the 95% control limits given below. Unit for urinary cadmium are ng/ml.

QC Mean Limits

Pool	UCL99	UCL95	Mean	LCL95	LCL99
base	0.553	0.501	0.338	0.176	0.125
476-1*	8.59	7.95	5.91	3.88	3.23

*Bench and blind control

Four pairs of blind split duplicates were included in the analyses for urinary cadmium, one member of each pair in each of the two runs. The table below shows the results on duplicate aliquots. All units for urinary cadmium are in ng/mL.

Pair	Run Number	Concentration 1	Concentration 2
1	1, 2	1.6	1.4
2	2, 1	0.3	0.3
3	2, 2	0.5	0.5
4	1, 1	0.6	0.4

Forty-five urine specimens were analyzed for beta-2-microglobulin in three analytical runs. Three samples from each of two bench control pools were included in each run. All means were within 95% control limits. The ranges for the 1436 pool in the first and second runs were outside the 99% limits; the range for the third run was inside the 95% limits. With four QC parameters being monitored, the probability of one or more being outside the 99% control limits for any given run is about 4%, which is larger than the 2% probability level used within the Division of Environmental Health Laboratory Sciences to warrant rejection of an analytical run. Consequently, the runs should be considered in control. Units below for beta-2-microglobulin are ug/l.

QC Mean Limits

Pool	UCL95	Mean	LCL95
1435	85.62	54.57	23.52
1436	295.15	222.23	149.51

Range of Triplicate Values

Within	Run
UCL99	UCL95
39.38	31.64
80.87	64.97

Eighty-five urine specimens were analyzed for creatine in two analytical runs. Two samples from each of two bench control pools were included in each run. Two samples of one of these pools were also included in each run as blind controls. For creatine, all means and ranges were within the 95% control limits shown below. Units for urinary creatine are mg/dl.

QC Mean Limits						Range of Duplicate Values, Within Run	
Pool	UCL99	UCL95	Mean	LCL95	LCL99	UCL99	UCL95
Urichem I	135.77	134.53	130.60	126.67	125.43	2.30	1.75
Urichem II*	96.42	95.34	91.90	88.46	87.38	4.31	3.28
*Bench and Blind Control							

Forty-five urine specimens were analyzed for total protein in two analytical runs. Two samples from each of two bench control pools were included in each run. Two samples of one of these pools were also included in each run as blind controls.

For urinary total protein, all means and ranges were within the 95% limits below except the first run mean and the second run range for one of the bench controls, which were outside the 99% limits. With six QC parameters, the probability of one or more being outside the 99% limits for a given run is about 6%, so the runs should be considered in control. Units for urinary total protein are mg/l.

QC Mean Limits						Range Limits	
Pool	UCL99	UCL95	Mean	LCL95	LCL99	UCL99	UCL95
Urichem II*	587.60	583.21	569.33	555.45	551.05	41.19	31.35
Urichem II (Diluted 1:4)	144.92	144.26	142.20	140.14	139.48	3.64	2.77
*Bench and blind controls							

Forty-five hair specimens were analyzed for lead and cadmium by the ICAP in four analytical runs. Five specimens of one hair-digest bench-control pool were included in each run. Both cadmium and lead are measured simultaneously by the ICAP, so 4 QC parameters are monitored, two means and two ranges. All means were within the 99% control limits given below except the mean for Pb in the third run, which was within the 99% control limits. All ranges were within the 95% limits shown below, except the range for Pb in the first run, which was outside the 99% limits. The probability of at least one of four QC parameters being outside the 99% limits in a given run is about 4%. By the multiple pool run accept/reject rules used within the Division of Environmental Health Laboratory Sciences, all four runs are considered in control. Units in the table below for are ug/g.

QC Mean Limits						Range of Quintuplet Values, Within Run	
Analyte	UCL99	UCL95	Mean	LCL95	LCL99	UCL99	UCL95
Cd	0.8087	0.7135	0.4126	0.1117	0.0165	0.3723	0.3124
Pb	4.494	4.165	3.126	2.087	1.758	1.858	1.559

The hair specimens were not large enough to run split duplicates to estimate run precision. Consequently, six pairs of duplicate hair digests from children of CDC employees were also included in the runs. The CV's for these duplicates averaged 13.5% for lead and 10.6% for cadmium.

Twenty-one hair digest samples were analyzed for arsenic by atomic absorption spectrophotometry. No certified reference materials were available for arsenic in hair to serve as bench or blind controls. Two EPA water quality QC samples were measured for arsenic. These are aqueous samples with dilute nitric acid, with target values of 20 and 40 ng/ml. Analytical results (mean of triplicate determinations) were 20.6 ng/ml and 35.9 ng/ml, respectively. Thus, although no quantitative quality control limits were available, the analysis gave values within 3.0% and 10.2% of the target values, respectively.

Three pairs of duplicate hair digests from digests from children of CDC personnel were also included to check run precision. All determinations of these were below detection limits (250 ng/g) for arsenic.

In summary, all of the analytical systems used for generating the data for this study appeared to be stable and in control.

RANGES OF VALUES FROM NORMAL INDIVIDUALS

The CDC laboratories analyzing the specimens provided the following information on ranges of values from normal individuals:

<u>Analyte</u>	<u>Range</u>
Blood lead	0 - 40 ug/dl (adults) <25 ug/dl (children)
Erythrocyte protoporphyrin	<35 ug/dl (<16 yr) <60 ug/dl (>16 yr)
Blood cadmium	0 - 5 ng/ml
Urinary arsenic	0 - 100 ng/ml
Urinary lead	0 - 100 ng/ml
Urinary cadmium	0 - 5 ug/ml
Urinary beta-2-microglobulin	0 - 250 ug/l
Urinary creatine	1 - 2 g/l/24 hr (males) 0.8 - 1.6 g/l/24 hr (females)
Urinary total protein	<135 mg/l
Hair lead	0.511 - 27.6 ug/g (males) 0.454 - 5.93 ug/g (females)
Hair cadmium	0 - 2.00 ug/g (males) 0 - 0.478 ug/g (females)
Hair arsenic	Values pending

The creatine normal ranges reported were based on a 24-hour urine sample. Since the specimens collected in this study were first morning voids, the normal ranges are not strictly applicable. In addition, values of urinary creatine less than 40 mg/dL are considered too low for purposes of normalizing other urinary analyte concentrations.

Appendix 21

Quality Assurance and Quality Control for Laboratory Analyses of Environmental Samples

1.0 Soils, Vacuum Dusts

Additional soil quality assurance measures were undertaken by MDHES because recovery information obtained by sending standard reference materials as blind field samples to EPA's contract laboratory showed poor recoveries by the Superfund laboratory. Table A shows precision and accuracy information for nine reference materials. Lead values, which were most essential in this study, gave overall recoveries (neglecting low standard concentrations¹) of 65.1%, 84.0% and 103.6% for VERSAR, MDHES-ICP, and MDHES X-ray, respectively. X-ray analyses of arsenic, copper, and zinc were adversely affected by a tungsten interference. After the samples were analyzed, it was discovered that a new tungsten-carbide grinding chamber had lost sufficient amounts of tungsten during the grinding of high silicate soils to interfere with the analyses of the above three elements. A correction for the above effect, though possible, had not been made during calibrations because the manufacturer had conjectured the grinder would sustain no loss of material.

Table B shows correlations for the eight elements of interest reported in this study for 29 field samples. A high correlation exists between MDHES ICP and X-ray results, as for most elemental comparisons. However, T-tests show that the arsenic, cadmium, silicon, and titanium results differ statistically between the methods. These differences were expected for arsenic because of the tungsten interference described above.

¹ East Helena soils were found to be high in lead concentrations--considerably higher than the background lead levels of most reference materials; comparisons against standards with elevated lead values were, therefore, appropriate.

2.0 Vegetables

Vegetation sample analyses were performed by MDHES using ICP/AAS instrument techniques. About 223 samples were dried, ground with a Wiley mill, digested by sealed bomb methods to prevent loss of volatile elements, and analyzed chemically.

Twenty-five samples were sent to an EPA Superfund contract laboratory by chain-of-custody procedures to provide sample security. Table C shows analytical comparisons between the MDHES and VERSAR, Inc., laboratories. Low t-values show the data to correlate favorably; i.e., each laboratory reports the same data for each element. However, large standard deviations here allow for this statistical data comparability. MDHES sent VERSAR several reference materials with the vegetable samples. Analytical recoveries based on these samples are listed in Table D, which compares precision and accuracy information for both laboratories. Reference samples were selected to present low, intermediate, and high elemental concentrations suitable to concentrations of vegetables analyzed in this study. The EPA sludge reference material lists what appear to be consensus values for the elements listed with large acceptable ranges; arsenic, for example, lists a mean of 17.0 ppm, with a range of 0 to 88.9, for the 95% confidence interval.

MDHES performance was superior overall to that of VERSAR. Any notable deviations in recoveries from 100% were found for those cases in which differences in small numbers tended to influence the average (overall) recovery in an artificial manner. For lead in vegetation, for this study, MDHES recovery was essentially 96% to 98%, VERSAR's recovery was 73% to 88%. Here again, recovery is a measure of laboratory accuracy.

3.0 Handwash Analyses

Laboratory recoveries and measures of instrumental accuracy were acceptable for handwash analyses. Lead recoveries averaged $100.8 \pm 6.6\%$, (N=57). Table E shows data for a limited number of blind repeat handwash analyses. Many ICP data points in the table reflect the detection limit problems noted above. Table F lists quality control comparisons for four EPA reference solutions sent to the MDHES laboratory as blind handwash samples. The MDHES analyzed each reference solution three times, with the results shown. EPA acceptance and warning limits are listed.

4.0 MDHES Chain-of-Custody Forms

See Forms A and B attached.

Table A

Precision and Accuracy Data for Soils Between Laboratories and Methods

Ref. No.	Reference Library Recovery	Al%	As (ppm)	Cd (ppm)	Cu (ppm)	Pb (ppm)	Si (%)	Ti (ppm)	Zn (ppm)
	NBS River Sed.	2.26+ 0.04	66	10.2+ 1.5	109+ 19	714+ 28			1720+ 170
1645	-VERSAR Inc.	0.389	37	5.0	97.8	520	26.9	105	1570
	% recovery	17.2	56.1	49.0	89.7	72.3			91.3
1645	-MDHES Lab (n=4)	2.34+ 0.02	64.9+ 1.7	20.4+ 5	112+ 6	680+ 55	20.6+ 7.3	426+ 9	1664+ 91
	% recovery	103.5	98.3	200	102.8	95.2			96.7
1645	-MDHES XRF (n=31)	1.98+ 0.088	67.3+ 4.7	4.3+ 3.4	266+ 26	709+ 6	22.34+ 0.18	1057.43	1706+ 45
	% recovery	87.6	102.0	42.2	244	99.3			99.2
	NBS Fly Ash		61+ 6	1.45+ 0.06	128+5	70+ 4		8600+ 1100	210+ 20
1633	-MDHES Lab (n=5)	12.8+ 0.5	64+ 3	N.D.	128+ 3	35+ 13	23+ 1	6181+ 68	232+ 20
	% recovery		104.9		100	50.0		71.9	110.5
1633	-MDHES XRF (n=3)	14.6+ 0.5	44+ 4	7.3+ 1.4	219+ 90	59+ 5	22.8 0.18	7264+ 128	384+ 161
	% recovery		72.1	503	171	84.3		84.4	183
	NBS Urban Part.	3.42+ 0.11	115+ 10	75+ 7	609+ 27	6550+ 80	12.52		4760+ 140
1648	-MDHES XRF (n=13)	3.48+ 0.13	125+ 5	59+ 7	661+ 18	7270+ 61	12.56+ 0.02	4546+ 60	4495+ 77
	% recovery	101.8	108.7	78.7	108.5	111	100.3		94.4
	USGS Andesite soil	9.07+ 0.18	0.84+ 0.27	0.061+ 0.008	60+ 6	36+ 5	27.67+ 0.27	6340+ 300	88+ 2
ACV1	-MDHES XRF (n=3)	9.35+ 0.30	N.D	7.2+ 3.7	124+ 52	47+ 3	27.51+ 0.24	6072+ 152	267+ 101
	% recovery	103.1		11803	207	130.6	99.4	95.8	303
	USGS Basalt	7.30+ 0.12	1.5	0.12	137+ 6	4+ 2	23.3+ 0.4	16000+ 500	102+ 7
BHVO	-VERSAR Inc.	0.443	0.5	0.05	67.8	0.6	34.1	1510	14.6
	% recovery	6.1			49.5	15	146.4	9.4	14.3
BHVO	-MDHES Lab (n=1)	5.1	1.1	0.11	130	13.8	15.9	12521	135
	% recovery	69.9	73.3	91.7	94.9	345	68.2	78.3	132
BHVO	-MDHES XRF (n=1)	7.61	6.8	2.25	164	7.5	23.8	15980	144
	% recovery	104.2	618	2045	119.7	188	102.1	99.9	141

Table A (Continued)

Precision and Accuracy Data for Soils Between Laboratories and Methods

Ref. No.	Reference Library Recovery	Al%	As (ppm)	Cd (ppm)	Cu (ppm)	Pb (ppm)	Si (%)	Ti (ppm)	Zn (ppm)
	USGS Diabase	8.14+ 0.06	1.2	0.1	84		24.2	6200+ 200	87+ 21
W2	MDHES Lab (n=3)	7.6+ 0.42	5.3+ 4.6	N.D.	111+ 5	11.8+ 0.7	21.7+ 0.8	5658+ 53	93+ 9.9
	% recovery	93.4	441.7		132		89.7	91.3	106.9
W2	MDHES XRF (n=1)	8.33	N.D.	1.4	116	26	23.0	6099	90
	% recovery	102.3		1400	138		95.0	98.4	103.4
	CANMET Blend		1296	467	11720	4604	21.3		59940
PDS02	MDHES Lab (n=2)	6.58+ 0.21	1407+ 66	493+ 103	12529+ 1127	4403+ 13	20.4+ 1.13	6590+ 110	60836+ 228
	% recovery		108.6	105.6	106.9	95.6	95.8		101.5
PDS02	MDHES XRF (n=8)	6.86+ 0.06	1149+ 27	443+ 6	10977+ 118	4425+ 66	21.96+ 0.03	6840+ 54	61887+ 1037
			88.7	94.9	93.7	96.1	103.1		103.2
	CANMET Soil	8.07+ 0.18	1.2+ 0.2	0.18+ 0.14	7+ 1	21+ 4	24.99+ 0.23	8600+ 200	124+ 5
S02	VERSAR Inc.	2.12	0.7	0.1	2.75	3.4	29.1	259	40.1
	% recovery	26.3	58.3	55.6	39.3	16.2	116.4	3.0	32.3
	EPA Soil	6.37	2070	52	2140	1310	27.0	2130	1730
4778	VERSAR Inc.	0.745	1800	35	2220	1400	36.9	290	1100
	% recovery	11.7	87.0	67.3	103.7	106.9	136.7	13.6	63.6
	MDHES Lab (n=3)	6.34+ 0.14	2049+ 149	9.46+ 149	2303+ 33	1247+ 106	30.76+ 1.25	1612+ 30	1481+ 137
	% recovery	99.5	99.0	18.2	107.6	95.2	113.9	75.7	85.6
	MDHES XRF (n=2)	6.73+ 0.02	2012+ 32	22+ 2	2185+ 10	1310+ 11	27.17+ 0.00	2521+ 40	1332+ 8
	% recovery	105.7	97.2	42.3	102.1	100.0	100.6	118.4	77.0
	Ave. VERSAR Rec.	15.3%	67.1%	57.3%	70.6%	52.6%	133.2%	8.7%	50.4%
			71.6%*	58.1%*		65.1%*			
	Ave. MDHES-LAB Rec.	91.6%	154.3%	103.9%	107.4%	136.2%	91.9%	79.3%	105.5%
			102.7%*	107.9%*		84.0%*			
	Ave. MDHES-XRF Rec.	100.8%	181.1%	1785%	148.0%	115.6%	100.1%	99.4%	138.0%
			93.7%*	64.5%*		103.6%*			

* See reference b. in Table 11 Summary

TABLE A SUMMARY

	Concentration Range E. Helena Study Soils ^a			Avg. Percent Soil Recoveries		
	<u>Min.</u>	<u>Mean</u>	<u>Max.</u>	<u>MDHES</u> <u>ICP</u>	<u>MDHES</u> <u>XRF</u>	<u>VERSAR</u>
Al (%)	1.7	6.6	12.8	91.6	100.8	15.3
As (ppm)	4.9	118	1,909	102.7 ^b	93.7 ^b	71.6 ^b
Cd (ppm)	0	20	160	107.9 ^b	64.5 ^b	58.1 ^b
Cu (ppm)	0.1	220	11,700	107.4	148.0	70.6
Pb (ppm)	3.1	527	7,965	84.0 ^b	103.6 ^b	65.1 ^b
Si (%)	12.8	30.0	37.1	91.9	100.1	133.2
Ti (ppm)	763	2939	35,600	79.3	99.4	8.7
Zn (ppm)	3	584	14,600	105.5	138.0	50.4

^a Soils considered were front/rear, side, and play.

^b Percent recoveries here are averages calculated without those recoveries associated with detection limit problems or problems in which percent recoveries were large because of differences between two small reported values.

TABLE B

MDHES LABORATORY QUALITY ASSURANCE COMPARISON BETWEEN AAS, ICP, AND XRF ANALYSES

OF 29 DUPLICATE SOIL MATERIALS FROM AREAS 1, 2, and 3

Elements	Mean	Std. Dev.	Mean Diff.	Corr.	2-Tail Prob.	T Value	Deg. Freedom	2-Tail Prob.
Al - Lab	65962	6781						
Al - XRF	65170	8184	792	0.773	0.000	0.82	28	0.420
As - Lab	51	46.0						
As - XRF	110	53.4	-59.7	0.478	0.009	-6.29	28	0.000
Cd - Lab	6.92	14.0						
Cd - XRF	20.9	14.1	-14.0	0.743	0.000	-7.49	28	0.000
Cu - Lab	141.9	216.3						
Cu - XRF	127.0	160.3	14.9	0.912	0.000	0.84	28	0.410
Pb - Lab	527.8	750.9						
Pb - XRF	485.6	624.2	42.2	0.970	0.000	1.08	28	0.288
Si - Lab	295310	26137						
Si - XRF	310403	22869	-15093	0.800	0.000	-5.14	28	0.000
Ti - Lab	2375	658						
Ti - XRF	2800	684	-425	0.884	0.000	-7.05	28	0.000
Zn - Lab	409.0	477.1						
Zn - XRF	441.0	455.6	-32.0	0.921	0.000	-0.92	28	0.365

TABLE C

Laboratory Quality Assurance Comparison Between Vegetation Analyses Done by MDHES and VERSAR

Elements	Mean	Std. Dev.	Mean Diff.	Std. Dev.	Corr.	2-Tail Prob	t-value	Deg. Freedom	2-Tail Prob.
As VERSAR	2.839	9.372							
As MDHES	3.704	11.867	-0.864	2.550	0.999	0.000	-1.40	16	0.182
Cd VERSAR	3.888	9.492							
Cd MDHES	8.858	16.934	-4.970	13.250	0.626	0.003	-1.68	19	0.110
Cu VERSAR	21.6725	51.769							
Cu MDHES	30.735	63.252	-9.062	34.645	0.837	0.000	-1.17	19	0.257
Pb VERSAR	43.650	118.373							
Pb MDHES	59.215	137.257	-15.565	33.009	0.977	0.000	-2.11	19	0.048
Zn VERSAR	90.990	151.247							
Zn MDHES	4672.15	20555.0	-4581.2	20476.6	0.521	0.018	-1.00	19	0.330

TABLE D

QUALITY ASSURANCE BLIND SAMPLE COMPARISON BETWEEN REFERENCE VEGETATION MATERIALS
AND EPA'S CONTRACT (SUPERFUND) LABORATORY AND MDHES RESULTS

	Al	As	Cd	Cu	Pb	Si	Ti	Zn
NBS 1571 ^a	--	10±2	0.11±0.01	12±1	45±3	--	--	25±3
VERSAR	60.3	6.5	0.54	11.3	33	N.D.	2.65	18.7
% Recovery	--	65	491	94.1	73.3	--	--	74.8
MDHES (n = 5)		11±1.5	0.37±0.15	13.2±1.9	43.4±7.2			28.6±9.5
% Recovery		110	336	110	96.4			114.4
NBS 1573 ^b	--	0.27±0.05	3	11.1	6.3±0.3	--	--	62±6
VERSAR	220	0.5	11.0	10.0	4.1	1.18%	8.8	53
% Recovery	--	--	367	90.1	65.1	--	--	85.4
MDHES	1200	0.32	2.0	9.0	4.0	0.85%	77	55
% Recovery	--	118.5	67	81.1	63.5	--	--	88.7
EPA Sludge	--	17.0 ^c	19.1	1080	526	--	--	1320
VERSAR	3070	2.5	68	1000	463	16.1%	70	1070
% Recovery	--	14.7	356	92.6	88.0	--	--	81.1
MDHES	12275	3.9	18.2	1050	514	11.3%	1875	1415
% Recovery	--	22.9	95.3	97.2	97.7	--	--	107.2
Avg. VERSAR Rec.	--	40	405	92.3	75.5	--	--	80.4
Avg. MDHES Rec.	--	83.8	166	96.1	85.9	--	--	103

^a Orchard leaves

^b Tomato leaves

^c EPA's: $x \pm t .95$ (df)s for As is 0 - 88.9 implying great uncertainty.

TABLE E

Results of Blind Repeat Analysis of Handwash Samples
East Helena Lead Study

Sample #	Al ^c	As ^c	Cd ^c	Cu ^c	Pb ^c	Si ^c	Ti ^c	Zn ^c
312-117-2-21-9 ^a	0.010 ^d	0.001	0.005 ^d	0.020	0.60	0.010 ^d	0.010	0.070
312-486-1-21-a ^b	0.052	-	0.005 ^d	0.005 ^d	0.025 ^d	-	0.005 ^d	0.072
312-001-1-21-1 ^a	0.020	0.003	0.005 ^d	0.080	0.130	0.010 ^d	0.005 ^d	0.240
312-484-5-21-7 ^b	0.037	0.002	0.005 ^d	0.067	0.068	-	0.005 ^d	0.234
312-051-2-21-0 ^a	0.020	0.0005 ^d	0.005 ^d	0.010	0.060	0.010 ^d	0.005 ^d	0.010
312-484-1-21-8 ^b	0.042	0.0005 ^d	0.005 ^d	0.005 ^d	0.058	-	0.005 ^d	0.012
312-151-2-21-9 ^a	0.020	0.001	0.005 ^d	0.010	0.025 ^d	-	0.005 ^d	0.060
312-482-1-21-6 ^b	0.010 ^d	0.001	0.005 ^d	0.010	0.025 ^d	-	0.010	0.060
312-221-1-21-0 ^a	0.020	0.0005 ^d	0.005 ^d	0.005 ^d	0.025 ^d	-	0.005 ^d	0.005 ^d
312-500-1-21-1 ^b	0.020	0.0005 ^d	0.005 ^d	0.005 ^d	0.025 ^d	-	0.005 ^d	0.005 ^d
312-145-1-21-9 ^a	0.020	0.0005 ^d	0.005 ^d	0.010	0.025 ^d	0.010 ^d	0.005 ^d	0.080
312-481-2-21-2 ^b	0.07	0.0005 ^d , 0.0005 ^d , 0.0005 ^d	0.005 ^d	0.005 ^d	0.025 ^d	-	0.005 ^d	0.076

^a Original sample number.^b Sample number assigned for repeat analysis.^c All values in ug/ml (ppm).^d These values are half (1/2) of the instrumental detection limit and were assigned to these samples by AQB when analytical results were below the instrumental detection limit.

TABLE F

Quality Control Comparison of Four EPA-Certified Reference Solutions Injected into MDHES Laboratory Sample Flow

	Al ^c	As ^c	Cd ^c	Cu ^c	Pb ^c	Ti ^c	Zn ^c
EPA Certified WP004(1) ^a	0.05	0.018	0.0015	0.008	0.018		0.012
MDHES 481-1-21-5/WP004(1)	0.09	0.019	0.005	0.005	0.025	0.005	0.005
MDHES 484-3-21-2/WP004(1)	0.08	0.018	0.005	0.010	0.025	0.005	0.010
MDHES 487-2-21-8/WP004(1)	0.14	0.017	0.005	0.005	0.025	0.005	0.005
EPA Acceptance Limits	0-.156	.0013-.0342	0-.0074	0-.0359	0-.0502		0-.0457
EPA Warning Limits	.0195-.126	.0055-.030	0-.00463	0-.0272	0-.0425		0-.033
EPA Certified WP004(2) ^a	0.450	0.060	0.013	0.050	0.120		0.080
MDHES 489-1-21-2	0.48	0.060	0.005	0.043	0.094	0.005	0.074
MDHES 489-2-21-a	0.43	0.059	0.010	0.050	0.110	0.010	0.070
MDHES 490-2-21-8	0.60	0.063	0.010	0.070	0.110	0.005	0.090
EPA Acceptance Limits	.263-.652	.0185-.101	.00594-.0199	.0191-.085	.0679-.172		.041-.126
EPA Warning Limits	.345-.569	.0294-.0904	.0091-.016	.0304-.0742	.0821-.158		.0556-.111
EPA Certified WP006(1) ^b	0.504	0.044	0.0325	0.0521	0.157	0.005	0.075
MDHES 491-1-21-1/WP006(1) ^b	0.546	0.043	0.030	0.047	0.162	0.005	0.179
MDHES 493-3-21-8/WP006(1) ^b	0.520	0.044	0.030	0.050	0.150	0.005	0.170
MDHES 495-2-21-2/WP006(1) ^b	0.574	0.041	0.028	0.051	0.114	0.005	0.182
EPA Acceptance Limits	.412-.582	.0258-.0615	.0243-.0369	.0438-.0593	.130-.181		.156-.192
EPA Warning Limits	.433-.560	.0303-.057	.0259-.0354	.0457-.0573	.136-.175		.161-.187
EPA Certified WP006(2)	0.730	0.235	0.039	0.339	0.43		0.418
MDHES 497-1-21-7/WP006(2)	0.779	0.120	0.036	0.334	0.442	0.005	0.416
MDHES 499-2-21-6/WP006(2)	0.718	0.120	0.040	0.340	0.420	0.005	0.408
MDHES 500-2-21-9/WP006(2)	0.736	0.225	0.035	0.345	0.408	0.005	0.409
EPA Acceptance Limits	.570-.913	.137-.329	.029-.045	.284-.387	.354-.511		.364-.467
EPA Warning Limits	.613-.870	.161-.305	.031-.043	.297-.374	.373-.492		.377-.455

^a Values in parentheses denote concentration of Standard: (1) = Concentration #1
(2) = Concentration #2

^b WP006(1) was diluted by a factor of 10 to produce a standard within the working range of the State Laboratory's ICP system. The certified values have been adjusted to reflect this.

^c All analytical results are in ug/ml (ppm).

CHAIN-OF-CUSTODY

~~Distribution: Original Accompanies Shipment; Copy to Originator Files~~

FORM B
CHAIN-OF-CUSTODY

Proj. Code	Project Name East Helena Lead Study		Area		Analysis	
TO: ANALYTICAL LAB		SAMPLE DESCRIPTION			REMARKS	
Sample No.	Dust	Soil	Vegetation	Other		
					<div style="writing-mode: vertical-rl; transform: rotate(180deg); position: absolute; top: 0; left: 0; width: 100px; height: 100px; border: 1px solid black; display: flex; align-items: center; justify-content: center; font-size: 10px;"> Pb Cd As Zn Cu </div>	

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)
Relinquished by: (Signature)	Date/Time	Received for Laboratory: (Signature)	Date/Time	Remarks	

Distribution: Original Accompanies Shipment; Copy to Coordinator and Files



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RTI/2474/79-01F

APPENDIX 22

SYSTEMS AND PERFORMANCE AUDIT OF THE
EAST HELENA LEAD EXPOSURE STUDY

by

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EPA Task Manager: W. F. Barnard
EPA Contract No. 68-02-3767
Work Assignment 79

Prepared for

Quality Assurance Division
Environmental Monitoring Systems Laboratory
U. S. Environmental Protection Agency
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February 1984

RESEARCH TRIANGLE PARK, NORTH CAROLINA 27709

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PREFACE

Since 1972 the Environmental Protection Agency (EPA) has been engaged in an on-site performance audit program of various monitoring groups throughout the United States and in several foreign countries. An on-site audit program is one part of an overall quality assurance program. The purposes of this audit program are twofold. First, agencies are furnished a means of rapid self-evaluation of the specific operation under study. The second objective of the program is to provide EPA with a continuing index of the validity of data reported to air quality data banks. The first, from a participant standpoint, is the more important. Used along with information obtained from an internal quality control program, the conclusions from the performance audits can be quite meaningful. However, results from any single set of audit data should not be construed as absolute indicators of data quality.

This program is being coordinated through the Quality Assurance Division (QAD) of the Environmental Monitoring Systems Laboratory (EMSL), Environmental Research Center, Research Triangle Park, North Carolina 27711. Comments or questions about the program should be sent to the above address.

SECTION 1 INTRODUCTION

The purpose of this report is to document the findings of the systems and performance audit evaluations of the office, laboratory, and field monitoring sites constituting the East Helena Lead Exposure Study located in Helena and East Helena, Montana. The audit was organized and coordinated through the Quality Assurance Division of the Environmental Protection Agency (EPA) Environmental Monitoring Systems Laboratory.

During the period spanning September 12 through September 15, 1983, personnel of the Research Triangle Institute (RTI) visited the East Helena Lead Smelter Exposure Study. Although the complete exposure study involves a wide variety of professional disciplines, RTI's audit was concerned only with the particulate sampling network. The sampling network consisted of nine high volume and two dichotomous samplers. The office, laboratory, and monitoring network constituting the East Helena Lead Exposure Study audit was conducted in accordance with recommendations given in the EPA publication Quality Assurance Handbook for Air Pollution Measurement Systems, Volume II, Ambient Air Specific Methods.

SECTION 2

SUMMARY

The results of the systems and performance audits are contained in this section. Section 2.1 summarizes the results of the systems audit, and Section 2.2 summarizes the results of the performance audit.

2.1 SUMMARY OF SYSTEMS AUDIT

The systems audit conducted at the East Helena Lead Smelter Exposure Study was divided into four major areas:

- o Review of the project documentation including the quality assurance and procedures manual.
- o Review of management organization, data archiving, and recordkeeping.
- o Chain of custody for samples, and
- o Monitoring site criteria evaluation.

Project documentation (Quality Assurance and Procedures Manual), specific to this study was not completely finished and was not available for inspection. The Environmental Program Manager reported that the monitoring program was still being modified, and as new information became available, the Quality Assurance and Procedures Manual would become final. During the interim, the State of Montana Air Monitoring Quality Assurance and Procedures Manual was being used as a reference for sampler operation. This manual was available and adequately covered all necessary aspects specified in the Quality Assurance Handbook for Air Pollution Measurement Systems, Volume I & II, including both high volume and dichotomous samplers. The State of Montana Air Monitoring Quality Assurance and Procedures Manual has been reviewed and approved by the EPA Region VIII Quality Assurance Coordinator.

The Air Quality Group of the Montana Department of Health and Environmental Sciences is well-organized and appears to work together satisfactorily. Data archiving and recordkeeping is organized and up-to-date. Data forms being used by the Air Quality Group satisfactorily document all information necessary to record valid samples. All data and recordkeeping are placed in cardboard file boxes and stored at the Air Quality Group's office for at least three years. The following individuals are responsible for the Montana Department of Health and Environmental Science particulate monitoring network data:

- o David Maughan - Environmental Program Manager
- o Jerry Schneider - Quality Assurance Coordinator
- o James Olsen - Air Monitoring Supervisor
- o Chuck Homer - Environmental Specialists
- o Diane Ertman - Laboratory Technician
- o Ben Myren - Contracted; Site Operator

A complete chain-of-custody has not been maintained for the particulate samples. Since the sample is handled by several people from the time of exposure, who was responsible for each sample at any one point in time is not recorded.

Monitoring site criteria have not been maintained at the Hastie Site. This site has been operating for several years and during this time a crab apple and several pine trees have grown enough to cause air flow obstruction. Several other sites are located in close proximity to East Helena streets, but because of the low volume of traffic, this is probably not a problem.

2.2 SUMMARY OF PERFORMANCE AUDIT

The East Helena Lead Exposure Study contained nine sites consisting of nine high volume and two dichotomous samplers. Table 1 summarizes the results of the flow rate audit conducted on these samplers. The last column to the right indicates each sampler's performance based on the criteria outlined in Section 5.

TABLE 1. SUMMARY OF PERFORMANCE AUDIT RESULTS FROM PARTICULATE SAMPLERS

SITE NAME AND SAROAD NUMBER	DATE OF AUDIT	SAMPLER TYPE	S/N	FLOW TYPE	*AUDIT SATISFACTORY/ UNSATISFACTORY
TOWNSEND SITE 27 0720 017 F05	9-13-83	High Volume	12733	N/A	S
SCHNEIDER SITE 27 0860 007 F02	9-13-83	High Volume	338	N/A	S
		Dichotomous	123	Total	S
				Coarse	S
				Fine	S
DUDLEY SITE 27 0860 725 F05	9-13-83	High Volume	12294	N/A	S
		Dichotomous	190	Total	S
				Coarse	S
				Fine	S
SOUTH SITE 27 0860 716 F02	9-13-83	High Volume	9378	N/A	S
DARTMAN FIELD SITE 27 0860 724 F02	9-13-83	High Volume	15708	N/A	S
HADFIELD EAST SITE 27 0860 719 F07	9-14-83	High Volume	139216	N/A	S
HADFIELD WEST SITE 27 0860 719 F02	9-14-83	High Volume	139218	N/A	S
FIRE HALL SITE 27 0860 714 F02	9-14-83	High Volume	16189	N/A	S
HASTIE SITE 27 0860 002 F02	9-14-83	High Volume	9091	N/A	S

* S = satisfactory; U = unsatisfactory

SECTION 3 AUDIT SYSTEMS VERIFICATION

Verification of the accuracy of RTI's particulate audit system was conducted prior to the audit trip. The particulate auditing system consisted of a Reference Flow (ReF) device and a dry gas meter. The verification of this system was conducted in RTI's Standards Laboratory on September 8, 1983. The accuracy of the ReF device used in auditing the high volume samplers (serial no. 272), was established through calibration with the RTI Roots meter, serial no. 7807141, a primary standard.

The Singer ten liter per revolution dry gas meter used in auditing the dichotomous samplers was calibrated in the RTI Standards Laboratory against a one cubic foot per revolution wet test meter (s/n 11AH2). Air flows were drawn simultaneously through the dry gas meter and the wet test meter with a vacuum pump. Flow rates through the meters were determined at least three times using an electronic timer for each air flow level generated. Measurements were based on five revolutions of the wet test meter (5.0 ft³). Temperature, pressure, and water vapor corrections were appropriately applied across both meters and incorporated into the calibration calculations.

The wet test meter is periodically calibrated at the EPA QAD/EMSL. The gasometer used to calibrate the wet test meter is a primary standard.

3.1 HIGH VOLUME SAMPLER FLOW MEASUREMENT

Instrument verified: Reference Flow (ReF) device

Serial No.: 272

Location: RTI Standards Laboratory

Date of verification: September 8, 1983

Calibrated by: R. Shores

Calibration Equation:

$$Q_{std} = 0.2645 (\Delta P P_a / T_a)^{1/2} + 0.1157$$

where:

Q_{std} = flow rate at standard conditions (temperature = 298.16 K; pressure = 760 mm Hg) in cubic meters/minute

ΔP = pressure drop across the Ref device in inches of water

P_a = atmospheric pressure (mm Hg)

T_a = atmospheric temperature in degrees Kelvin (273.16 + °C)

3.2 DICHOTOMOUS SAMPLER FLOW MEASUREMENT

Instrument verified: 10 liter per revolution dry gas meter

Serial No.: 80SH583660

Location: RTI Standards Laboratory

Date of verification: September 8, 1983

Calibrated by: R. Shores

Calibration Equation:

$$Q_{std} = 0.9343 (Q_{ind}) [(298.16 \times P_a) / (760 \times T_a)]$$

where:

Q_{std} = flow rate at standard conditions (temperature = 298.16 K; pressure = 760 mm Hg) in liters/minute.

Q_{ind} = flow rate that is indicated by the dry gas meter at meter conditions.

P_a = atmospheric pressure (mm Hg)

T_a = atmospheric temperature (K)

0.9343 = correction factor for dry gas meter versus standard wet test meter (s/n 11AH2)

SECTION 4

AUDIT PROCEDURES

Systems audit procedures have been determined in accordance with recommendations given in Environmental Protection Agency's (EPA's) Quality Assurance Handbook for Air Pollution Measurement Systems, Volume II. Performance audit procedures for particulate samplers consist of an audit of the samplers inlet flow rate. Sections 4.1 and 4.2 discusses the audit procedures used during the audit for the systems and performance audit, respectively.

4.1 SYSTEM AUDIT APPROACH

The systems audit of the office, laboratory, and field sites of the East Helena Lead Smelter Exposure Study was conducted in accordance with the recommendations given in the EPA publication Quality Assurance Handbook for Air Pollution Measurement Systems, Volume II, emphasizing the guidelines for systems audits given in Section 2.0.11, "Systems Audit Criteria and Procedures for Ambient Air Monitoring Programs."

The approach taken in conducting the systems audit followed these steps:

- o Review of the project's documentation including quality assurance and procedures manual,
- o Review of management organization, data archiving, and record-keeping,
- o Chain of custody for samples, and
- o Monitoring site criteria evaluation.

4.2 PERFORMANCE AUDIT APPROACH

The performance audits of the field sites constituting the East Helena Lead Smelter Exposure Study were conducted in accordance with the recommendations given in the EPA publication Quality Assurance Handbook for Air Pollution Measurement Systems, Volume II, emphasizing the guidelines for performance audits given in Section 2.0.12, "Audit

Procedures for Use by State and Local Air Monitoring Agencies" and a draft version of the EPA document entitled, Inhalable Particulate Network Operations and Quality Assurance Manual, March 1983. The specific approach taken for the two types of samplers audited are described in the following subsections.

4.2.1 High Volume Samplers

A Reference Flow (ReF) device was used to audit the flow rate calibration of the high volume particulate samplers. The ReF device is a limiting orifice device which yields audit checks of sampler flow rate by measuring the pressure drop across the orifice using a water manometer. This pressure drop is translated into a flow rate at either standard or actual conditions using the applicable calibration equation. Traceability is established by calibrating the ReF device using a Roots meter, a primary standard.

4.2.2 Dichotomous Samplers

A dry gas meter was used to measure the total and fine flow rates and the coarse flow rate was determined as the difference between the total and fine flow rates. After clean filters were installed and the rotameters were properly set the dry gas meter was connected to the sampler's inlet. An actual flow rate was obtained by measuring a volume of air over an interval of time and applying the appropriate calibration factor for that meter. A total flow rate was obtained first, and by capping off the coarse flow vacuum line under the virtual impactor (and filter holder), a fine flow rate was obtained. The coarse flow rate was determined by subtracting the fine flow rate from the total flow rate.

SECTION 5

AUDIT RESULTS

During the period of time spanning September 13 through September 15, 1983, RTI personnel conducted systems and performance audits at eight sites surrounding the American Smelting and Refining Company (ASRCO) located in East Helena, Montana.

Data for each portion of the audit are presented in this section. Each component of the systems audit, as described in Section 4, is discussed in Section 5.1. The particulate samplers' audit data are compared in terms of percent difference of flow rate. The particulate sampler audit results are given in Section 5.2. The following criteria based on average percent difference in flow rate may be used as a measure to evaluate the performance of a particulate sampler:

- (1) Satisfactory = $|\text{Percent Difference}| \leq 10$ percent
- (2) Unsatisfactory = $|\text{Percent Difference}| > 10$ percent

5.1 SYSTEMS AUDIT RESULTS

Project documentation (quality assurance and procedure manual) specific to this study was not available for inspection. The State of Montana Environmental Program Manager reported that the monitoring program was still being modified, and as new information became available, the quality assurance and procedures manual would become final. During the interim, the State of Montana Air Monitoring Quality Assurance and Procedures Manual was used as a reference for sampler operation. This manual was available and adequately covered all necessary aspects specified in the Quality Assurance Handbook for Air Pollution Measurement Systems, Volume I & II, including both high volume and dichotomous samplers. The State of Montana Air Monitoring Quality Assurance and Procedures Manual has been reviewed and approved by the EPA, Region VIII Quality Assurance Coordinator.

Figure 1 represents the chain-of-custody being maintained for filters/samples collected as part of the East Helena Lead Smelter Exposure Study. However, this chain-of-custody is not being documented. From the time of exposure, the sample is handled by several people and who was responsible for the sample at any one point in time is not recorded. The hi-vol data record form is placed with the filter prior to exposure and remains with the filter thereafter. Because the chain-of-custody becomes important after the filter has been exposed, the hi-vol data record form could also provide the necessary chain-of-custody. To provide this chain-of-custody, the hi-vol data record form should include a sign-in and out of possession section. This section would provide for initials, date and time, both in and out of possession for each set of initials. This section would provide each sample a record of who was responsible for that sample from exposure to data archiving. Strips are cut from samples for further chemical analysis. The same hi-vol data record form can be copied and the copy could then become the strip's chain-of-custody form. Figure 2 represents a hi-vol data record form which remains with a sample from exposure to data archiving. This form establishes the link between serial numbers stamped onto each filter and the SAROAD identification number, flow rate, and date of the sample. Each filter serial number is also recorded in the filter weight log which contains the sample collection information and the filter weight information.

The chain-of-custody procedure presently being maintained for sample collected as part of the East Helena Lead Smelter Study will not affect the quality of the samples. However, this chain-of-custody does allow for the potential of tampered samples with no specific individual being responsible.

5.1.1 Townsend Site (Visited 9-13-83)

One high volume sampler, which is bolted to a four feet by eight feet piece of plywood mounted on scaffolding six feet above the ground is located at the Townsend Site, is located fifty-five feet southwest of Townsend road and twenty-one feet east of the Artisan Furniture

Filter Preparation
(Laboratory)

- C. Homer
- o Receives Filters
 - o Q/C Conducted
 - o Deliver to D. Ertman

- D. Ertman
- o Receives Filters
 - o Filter Conditioning
 - o Determine Tare Weight
 - o Documentation
 - o Deliver to C. Homer

- C. Homer
- o Receives Filters
 - o Ship to Field Site

Filter Exposure
(Field Site)

- Site Operator
- o Receives Filters
 - o Initial Entry of Data on data form (See Figure 2)
 - o Filter Exposure
 - o Complete Entry of Data on Data Form
 - o Deliver Sample to C. Homer

Post Exposure Weighing
(Laboratory)

- C. Homer
- o Receives Samples
 - o Q/C Conducted
 - o Calculation of Total Air Volume Sampled
 - o Deliver to D. Ertman

- D. Ertman
- o Receives Samples
 - o Filter Conditioning
 - o Determine Gross and Net Weights
 - o Calculate Concentration ($\mu\text{g}/\text{m}^3$)
 - o Cuts Sample Strips for Further Chemical Analysis
 - o Deliver to C. Homer

- C. Homer
- o Receives Samples
 - o Archives Samples in Storage Boxes
 - o Tabulates Data

FIGURE 1. CHAIN OF CUSTODY, PARTICULATE FILTERS, STATE OF MONTANA

[illegible]

This image shows a single sheet of white paper with horizontal blue or grey ruling lines. The lines are evenly spaced and run across the width of the page. There is no handwriting or other markings on the paper.

Operator _____		
Filter No. _____	Sampler No. _____	
Date _____	Time or Meter Reading _____	Flowmeter Reading _____
Start: _____		
End: _____		

Start: WIND VIS. SKY HUM. TEMP. PRESSURE

Codes*
WIND: 1-calm 2-light 3-gusty 4-strong
VISIBILITY: 5-clear 6-hazy
SKY: 7-clear 8-scattered 9-overcast
HUMIDITY: A-dry B-moderate C-humid D-rain or snow
TEMP. (°F): E:<20 F:20-40 G:41-60 H:61-80 I:>80

Unusual activities or conditions near the sampling site:

Particulate Concentration	$\mu\text{C}/\text{M}^3$
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10
11	11
12	12
13	13
14	14
15	15
16	16
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100	100

12

Upholster building. Southeast of the site, twenty-one feet away is a barn-like building that is four-to-five feet above the sampler; however, this structure should not cause any air flow obstruction, because it is relatively small (approximately 10 ft x 20 ft). The only point source in close proximity is a wood stove chimney in the upholstery building. Because the chimney is so close to the sampler, a biased sample could result in periods with certain meteorological conditions. The surrounding area may be described as a subdivision neighborhood with paved streets.

5.1.2 Schneider Site (Visited 9-13-83)

One high volume sampler is located at the Schneider site. The dichotomous sampler located at this site is inside the Schneider residence and is part of an indoor pollution study. The high volume sampler is bolted to a four feet by eight feet piece of plywood and is mounted on scaffolding six feet above the ground. The site is sixty feet southeast of Tejon Lane and one hundred and twenty feet southwest of the Schneider residence. The only air flow obstruction may be caused by two two-story colonial style homes which are at least one hundred feet away from the site. The only point sources in close proximity to the site are wood stove chimneys. The surrounding area may be described as a subdivision neighborhood with paved streets.

5.1.3 Dudley Site (Visited 9-13-83)

One dichotomous and one high volume sampler are located at the Dudley site. The high volume sampler is bolted to a four feet by eight feet piece of plywood and is mounted on scaffolding six feet above the ground. The dichotomous sampler was mounted on two inch by four inch planks extended off one edge of the platform approximately two feet, with a distance between the two samplers of six feet. This site is located on the back property line of a one-half acre lot. A one story ranch house is located forty-five feet north of the site, and the site is one hundred and twenty-five feet south of Dudley Road. South and southwest of the site are vacant lots, overgrown with weeds. The

surrounding area may be described as a subdivision neighborhood with paved streets.

5.1.4 South Site (Visited 9-13-83)

One high volume sampler, which is bolted to a four feet by eight feet piece of plywood mounted on scaffolding; six feet above the ground is located at the South site. This site is located only two thousand feet southeast of the Lead Smelter. The surrounding area is open fields and scrub brush with no air flow obstructions.

5.1.5 Dartman Site (Visited 9-13-83)

One high volume sampler bolted to a four feet by eight feet piece of plywood mounted on scaffolding six feet above the ground is located at the Dartman site. This site is located in a field one hundred and fifty feet west of a lightly traveled dirt road. East of the site a row of trees borders the field one hundred and twenty-five feet away. The area is a combination of pastures, overgrown pastures, and houses with no air flow obstructions.

5.1.6 Hadfield East and West Site (Visited 9-14-83)

Two high volume samplers, bolted on opposite ends of a six feet by twelve feet platform which is mounted on scaffolding ten feet above the ground, are located at this site. The sampler mounted on the east side of the platform is designated "East," and the sampler mounted on the west side is designated "West". This site is located thirty-six feet southwest and seventy-two feet southeast of Cleveland Avenue and Main Street, respectively. This site is surrounded by buildings, but because of the height of the sampler there is no air flow obstruction. This site is approximately five hundred and fifty-feet northwest of the lead smelter.

5.1.7 Hastie Site (Visited 9-14-83)

One high volume sampler which is bolted on a four feet by six feet piece of plywood mounted on scaffolding fifteen feet above the ground

is located at this site. This site is located approximately four hundred and twenty feet northeast of the lead smelter. A crab apple and several pine trees have overgrown this site, causing severe sitting criteria violations. The trees cover one ninety degree gradient eleven to twenty-three feet from the sampler and three to ten feet above the sampler. Because the trees are directly between the sampler and the lead smelter, collected ambient air concentrations may be lower than surrounding concentrations. This site has only recently been overgrown by the crab apple and pine trees. The impact of the trees upon the collected ambient air data may best be evaluated through comparison to the past years of data collected at this site.

5.2 PERFORMANCE AUDIT RESULTS

Eight high volume and two dichotomous samplers were audited. Table 1 contains the audit results for the high volume samplers, and Table 2 contains the audit results for the dichotomous samplers.

TABLE 2. HIGH VOLUME SAMPLER PERFORMANCE AUDIT RESULTS

Site Name (Date of Audit)	Serial Number	Plate Number	Audit Flow (std. m ³ /min)	Sampler Flow (std. m ³ /min)	Difference		
					std. m ³ /min	Percent	Avg.
TOWNSEND (9-13-83)	12733	NP	1.707	1.650	-0.057	- 3.3	-5.1
		18	1.482	1.415	-0.067	- 4.5	
		13	1.371	1.312	-0.059	- 4.3	
		10	1.263	1.194	-0.069	- 5.5	
		7	1.026	0.945	-0.081	- 7.9	
SCHNEIDER (9-13-83)	338	NP	1.682	1.659	-0.023	- 1.4	-2.5
		18	1.465	1.435	-0.030	- 2.0	
		13	1.352	1.323	-0.029	- 2.1	
		10	1.227	1.195	-0.032	- 2.6	
		7	1.017	0.972	-0.045	- 4.4	
DUDLEY (9-13-83)	12294	NP	1.470	1.408	-0.062	- 4.2	-4.9
		18	1.294	1.233	-0.061	- 4.7	
		13	1.195	1.131	-0.064	- 5.4	
		10	1.102	1.044	-0.058	- 5.3	
		7	0.915	0.870	-0.045	- 4.9	
SOUTH (9-13-83)	9378	NP	1.473	1.486	+0.013	+ 0.9	-4.8
		18	1.298	1.288	-0.010	- 0.8	
		13	1.185	1.152	-0.033	- 2.8	
		10	1.092	1.015	-0.077	- 7.1	
		7	0.923	0.794	-0.129	-14.0	

TABLE 2. HIGH VOLUME SAMPLER PERFORMANCE AUDIT RESULTS
(cont'd)

Site Name (Date of Audit)	Serial Number	Plate Number	Audit Flow (std. m ³ /min)	Sampler Flow (std. m ³ /min)	Difference		
					std. m ³ /min	Percent	Avg.
DARTMAN FIELD (9-13-83)	15708	NP	1.470	1.441	-0.029	- 2.0	-5.4
		18	1.302	1.253	-0.049	- 3.8	
		13	1.204	1.159	-0.045	- 3.7	
		10	1.089	1.034	-0.055	- 5.1	
		7	0.911	0.800	-0.111	-12.2	
HADFIELD EAST (9-14-83)	139216	NP	1.482	1.434	-0.048	- 3.2	-7.0
		18	1.305	1.233	-0.072	- 5.5	
		13	1.219	1.116	-0.103	- 8.4	
		10	1.103	0.982	-0.121	-11.0	
HADFIELD WEST (9-14-83)	139218	NP	1.396	1.468	+0.072	+ 5.2	-5.5
		18	1.332	1.257	-0.075	- 5.6	
		13	1.234	1.143	-0.091	- 7.4	
		10	1.127	1.030	-0.097	- 8.6	
		7	0.942	0.836	-0.106	-11.3	
FIRE HALL (9-14-83)	16189	NP	1.374	1.316	-0.058	- 4.2	-7.2
		18	1.239	1.143	-0.096	- 7.7	
		13	1.164	1.044	-0.120	-10.3	
		10	1.067	0.995	-0.072	- 6.7	
HASTIE (9-14-83)	9091	NP	1.403	1.444	+0.041	+ 2.9	+4.2
		18	1.236	1.293	+0.057	+ 4.6	
		13	1.154	1.205	+0.051	+ 4.4	
		10	1.065	1.116	+0.051	+ 4.8	

TABLE 3. DICHOTOMOUS SAMPLER PERFORMANCE AUDIT RESULTS

Site Name (Date of Audit)	Sampler Model and S/N	Flow Type	Audit Flow (act. L/min)	Sample Flow (act. L/min)	Difference	
					Act. L/min	Percent
SCHNEIDER (9-13-83)	Sierra S/N 123	TOTAL	16.87	16.67	-0.20	- 1.2
		FINE	15.26	15.00	-0.26	- 1.7
		COARSE	1.61	1.67	+0.06	+ 3.7
DUDLEY (9-13-83)	Sierra S/N 190	TOTAL	15.65	16.67	+1.02	+ 6.5
		FINE	14.09	15.00	+0.91	+ 6.5
		COARSE	1.56	1.67	+0.11	+ 7.1

APPENDIX 23

ESTIMATING THE AMOUNT OF SOIL INGESTED BY YOUNG CHILDREN
THROUGH TRACER ELEMENTS

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ABSTRACT

In this pilot study, we modified methods used in estimating the amount of soil ingested by ruminants to measure soil ingested by children. Using aluminum, silicon, and titanium as tracers, we estimated soil ingestion for 59 children aged 1-3 years from East Helena, Montana. Estimated daily soil ingestion based on aluminum and silicon concentrations were 121 and 184 mg/day, respectively; the estimate based on the titanium concentration was about 10 times higher, 1,834 mg/day. We do not consider these estimates accurate measures of soil ingestion, although the method we used is a reasonable approach that, to our knowledge, has not been used before for humans. Refinement of this method and a better understanding of the metabolism of aluminum, silicon, and titanium will lead to more accurate estimates of soil ingestion in toddlers.

BACKGROUND

An assessment of the human health risk associated with toxic materials in the environment is only as good as the estimate of exposure. Relatively "good" estimates are available for the amount of air breathed or water ingested. However, estimates of the amount of dirt and dust ingested during normal activities are based on little objective evidence, and they vary widely. This study estimates soil ingestion in children by measuring aluminum, silicon, and titanium in soil, dust, and feces.

Previous estimates of soil ingestion in children

Previous estimates of the soil ingestion of children have generally been guesses or a combination of guesswork and measurements. Lepow et al. measured a mean of 10 milligrams (mg) dirt on the hands of 22 children. They guessed that a child puts its hands into its mouth approximately 10 times a day for a daily soil intake of 100 mg/day.¹ The National Research Council estimated that young children ingest an average of 40 mg/day of street dust.² Day et al. measured 5-50 mg of dirt transferred from a child's hand to a sticky sweet and estimated that a daily intake of 2-20 sweets would lead to a dirt intake of 10-1,000 mg.³ Kimbrough et al. estimated that the average amount of soil ingested per day is 1 gram (g) for children aged 9 to 18 months, 10 g for 18 to 42 months, 1 g for 42 months to 5 years, and 100 mg for 5 years through adulthood.⁴

Estimates of soil ingestion in ruminants

Veterinary scientists have developed methods for measuring soil ingestion by grazing animals. Using nonabsorbed elements as tracers, they have measured fecal excretion of elements that have high concentrations in soil. Silicon and titanium have usually been used for this purpose, and estimates of soil ingestion in ruminants, such as cows, have ranged from .42 to 4219 g/day.^{5,6} We adapted this method for estimating the amount of soil ingested by children.

METHODS

Collection and processing of stool samples

As part of a health study of residents living near a lead smelter in East Helena, Montana, during the summer of 1984, we collected 3-day fecal specimens from 70 children ages 1 to 3 who were not toilet trained. Parents participating in the stool collection part of the study were given a free, 3-day supply of one brand of disposable diapers. Parents placed all used diapers in large plastic bags for daily collection by study personnel. They were also asked to wipe the child's bottom with the diaper itself and to refrain from using creams or lotions on the child's bottom for the 3-day collection period.

Stool was scraped from the diaper liner and put in a clean plastic bottle. The diaper liner was separated from the diaper and cleaned in distilled water to remove adherent stool. The rinse water was added to the stool material in the plastic bottle, and the bottles were stored in a walk-in refrigerator at 4°C for several months before being freeze-dried. Some of the samples required additional drying in an oven. The stool samples were then inspected. Hair, small pieces of white plastic material (thought to originate from the diaper cleaning process), and raisins were the only identifiable objects. These were removed, and the samples were weighed and then

homogenized to a fine powder in a food blender with stainless steel blades. (Neither an SPEX shatterbox nor an herb grinder worked adequately.) The powder was pressed to 5-g pellets and analyzed for aluminum, silicon, and titanium by x-ray fluorescence (XRF). (Since no stool reference standards exist and since no National Bureau of Standards biological reference materials contain silicon or titanium and few contain aluminum, the XRF results could not be verified. Reagent-grade stock solutions can be used to calibrate inductively coupled plasma atomic emission spectrometry (ICP). For this reason, ICP analysis was performed on fecal material remaining after the pellets were made. Quality assurance was assessed by adding precise quantities of aluminum, silicon, and titanium to stool and assessing recovery (Table 1)).

Collection and processing of soil and dust samples

In addition to stool samples, yard soil and indoor dust samples were collected from most of the participating children's houses. After sod and grass were removed, four soil samples from the front yard and one from the back were obtained from the top 1 inch of ground with a stainless steel corer and were composited in the field. Grab dust samples were collected from vacuum cleaner bags in participants' homes. Aluminum, silicon, and titanium concentrations in soil and housedust were analyzed by XRF; reference materials are available for XRF analysis of these elements in soil and housedust.

Algorithm for determining soil ingestion

Estimates of daily soil ingestion for each child i ($M_{i,e}$) based on aluminum, on silicon, and on titanium ($M_{i,e}$) were calculated according to the following formula:

$$M_{i,e} = \frac{f_{i,e} \times F_i}{s_{i,e}}$$

where $M_{i,e}$ = estimated soil ingestion for child i based on element e in mg/day,

$f_{i,e}$ = concentration of element e in fecal sample for child i in mg/g

F_i = daily fecal dry weight for child i in g/day, and

$s_{i,e}$ = concentration of element e in child i 's yard soil in mg/g

Because stool weights in this study were so much less than expected (as low as 1.8 g/day), F_i was replaced with 15 g/day, an estimate of the daily fecal dry weight for all children in this age group. This estimate was derived from reanalysis of data presented in a 1979 paper.⁷ (Seventeen infants aged 13-24 months produced a mean of 1.7 stools per day. Average stool weight was 35 g, of which 73.8% was water. The calculated mean dry weight of the stools was approximately 15 g/day.)

In addition to aluminum, silicon, and titanium that children ingest as part of their normal diets, certain materials provide concentrated sources of these elements. For example, baked goods can contain large amounts of aluminum, beer is a saturated solution of silicon, and paint contains large quantities of titanium. If a child has consumed a concentrated source of one element, the estimated amount of soil ingested based on that element will be higher than the estimates based on the other two elements. An alternative method of estimating soil ingestion is to use the minimum of the three soil ingestion estimates for each child. Using the same notation as before, according to this method, we find:

$$M_i = \text{Minimum } (M_{i,e})$$

where M_i = estimated soil ingestion for child i in mg/d, and

$M_{i,e}$ = estimated soil ingestion for child i based on element e in mg/d.

Questionnaire

A questionnaire was administered as part of the Childhood Lead Study. It included questions about demographic information and childhood habits (see Montana Cooperative Agreement Report). An additional questionnaire was administered to parents of participants in this soil ingestion study. It included questions about foods eaten during the study by participating children and activities during the days of the study.

RESULTS

Study population

Three-day stool samples and questionnaires were obtained from 70 children. Labels on 2 stool samples were lost; the 68 remaining samples were analyzed by XRF. Sixty-five of these samples were also analyzed by ICP. (Three samples were lost after XRF was completed.) Unless otherwise noted, all statistics in this report describe these 65 children. The mean age of children studied was 1.6 years. Sixty-five percent were male (Table 2). There was no significant difference in age between males and females.

Stool samples

Mean and median daily fecal dry weight were 7.3 grams/day and 6.7 grams/day, respectively (Table 3). Sixty-five stool samples were analyzed by both XRF and ICP (in the absence of suitable reference materials). The correlation between the two methods is good for silicon and titanium but poor for aluminum (Table 4, Figures 1-3). Analysis of the residuals revealed no systematic errors. Unless otherwise stated, the ICP values are used in the analyses that follow.

The mean, median, and geometric mean fecal concentrations of aluminum, silicon, and titanium are shown in Table 5. The distributions of elements in the 65 stools are highly skewed; only silicon appeared to be normally distributed with logarithmic transformation.

Soil and dust samples

Front and back yard composite soil samples were obtained for 59 of the 65 children with ICP stool data; dust samples were available for 45 children. Both soil and indoor vacuum grab sample data were available for 42 of these children (Table 6).

Mean, median, and geometric mean soil and dust concentrations and standard deviations are shown in Table 7. Soil silicon and titanium and dust titanium concentrations were log normally distributed. Correlations between yard soil and indoor dust aluminum, silicon, and titanium concentrations were .14, .18, and .04, respectively.

Estimated soil ingestion by individual elements

Estimates of soil ingestion based on aluminum, silicon, and titanium were calculated for the 59 children with both composite yard soil and ICP data (Table 8, Plots 4, 5, and 6). The arithmetic mean of soil ingestion estimates based on aluminum (181 mg/day) is similar to the one based on silicon (184 mg/day), but the estimate based on titanium (1,834 mg/day) is tenfold higher. As would be expected, the arithmetic mean estimate based on the minimum of the three estimates for each child (108 mg/day) is lower than that calculated on any individual element (Table 9, Plot 7).

Soil ingestion estimates calculated with mean soil element concentrations

Soil ingestion estimated with arithmetic mean soil concentrations of aluminum, silicon, and titanium in all the children's yards (Table 10) is not appreciably different from soil ingestion estimated with the concentrations in each child's yard (Table 10). Estimates of soil ingestion based on the geometric means of soil element concentrations will be lower than those based on the arithmetic means.

Analysis of dust ingestion estimates

Table 11 shows the estimates of dust ingestion obtained when one assumes that all of each element present in stool derives from housedust only. Because concentrations of aluminum, silicon, and titanium in dust tended to be lower than concentrations in soil, estimates of dust ingestion are higher than estimates of soil ingestion. In this analysis, the relative amounts of dust and soil ingestion cannot be estimated.

Questionnaire data

There was no apparent relationship between responses to questions about childhood behaviors that might be associated with increased soil ingestion, such as furniture mouthing, paint chip ingestion, etc., and estimated soil ingestion by any of the above methods. Dietary histories obtained by questionnaire were not complete enough for meaningful analysis.

DISCUSSION

Soil ingestion estimates

On the basis of our data and calculations, we estimated that mean daily soil ingestion in the East Helena children is between 108 mg/day (based on the minimum estimate of all elements for each child) to 1,834 mg/day (based on titanium). The estimates of soil ingestion for the children based on aluminum and silicon are an order of magnitude less than the estimate based on titanium. To determine which estimate best represents the amount of soil a child ingests, we would have to know which element is the best tracer for soil ingestion---and that information is not available.

It is difficult to explain why the soil ingestion estimate based on titanium is so much larger than that for either of the other elements. Although titanium is present in paint, houses in East Helena did not have chipping or peeling paint. One would expect the estimate from aluminum to be highest, since aluminum occurs so commonly in foods. Titanium does not occur in large quantities in drinking water or foods, including milk,⁸ and it is present in children's vitamins in only minute quantities (personal communication, Squibb Pharmaceutical Company). It is present in some toothpastes, especially gels, as the ingredient of lowest concentration (<2.5%); however, the exact amounts of titanium in toothpaste are proprietary information (personal communication, Colgate-Palmolive Company). Titanium does not leach from diapers (unpublished data, Center for Environmental Health, Centers for Disease Control) and is not present in baby powder (personal communication, Johnson and Johnson Company).

It is unlikely that stool samples were contaminated with titanium during processing. (We were unable to elute titanium from plastic gloves, sample jars, and similar items.) We think it likely that a combination of differences in absorption or metabolism of these elements in children compared with adults and unrecognized sources of titanium (in diet or in the laboratory processing of stools) are responsible for the relatively high soil ingestion estimate based on titanium.

In addition to the assumption that aluminum, silicon, and titanium are neither introduced into nor lost from samples during processing and that all urine and feces were completely removed from the diapers, we needed to make several other assumptions in applying our algorithm (Table 12). These are summarized below.

Sources of ingested soil and dust

The algorithm assumes that children ingest predominantly soil from their own yards and that concentrations of elements in composite front and back yard soil samples are representative of overall concentrations in the yard. In East Helena, the calculation of soil ingestion with the study population's mean yard soil concentrations of elements (Table 11) instead of each child's individual values (Table 9) had little effect on estimated soil ingestion; in other areas this assumption may be more important.

Because garages, cars, and places in the house that are inaccessible to children may be vacuumed with a household vacuum cleaner, grab samples may not be representative of dust that children may ingest. The magnitude of housedust ingestion, like that of soil ingestion, is unknown. In East Helena, dust concentrations of elements (from vacuum cleaner grab samples) tended to be lower than soil concentrations. Thus, if all of a child's intake of a given element came from dust, he or she would need to eat much more dust than soil to achieve a given fecal excretion. Children probably eat a combination of soil and dust. In East Helena, the algorithm used, which does not distinguish between soil and dust ingestion, will, if dust ingestion is ignored, overestimate the amount of soil ingestion.

Fecal weight

Dry fecal sample weights in this study were much lower than we had expected. Some of the reported fecal weights were so low that they were hard to accept as valid (Table 4). Although we reviewed the diaper processing procedure and could not identify any step at which stool samples were lost, it is possible that not all of the stool was recovered from the diapers or that some specimens were misplaced before the weighing. It is also possible that parents did not submit all soiled diapers.

Soil ingestion calculated by the algorithm is directly proportional to fecal weight. Because evidence that the fecal sample weight data were inaccurate, we chose to use an estimate of 15 grams per day as the average fecal weight in toddlers. (See page 7 for how this number was derived.) In reality, daily fecal weight varies among toddlers; use of a single fecal weight estimate for all children is a potential major source of bias in the calculated ingestion estimates.

Metabolism and dietary sources of aluminum, silicon, and titanium

Studies have shown that aluminum, silicon, and titanium can be absorbed from the gastrointestinal tracts of adults, although usually in small amounts.⁸⁻¹⁴ Studies of lead metabolism show that absorption of elements may be significantly different in children and adults.² Unless young children were in negative metabolic balance for these elements, however, absorption of these elements in urine did not lead to an underestimate of soil ingestion in our study. In children who are still wearing diapers, mixing of urine and feces will decrease the importance of gastrointestinal absorption on soil ingestion estimates obtained by using the algorithm. (Studies in older children and adults will need to take the possibility of absorption and urinary excretion into account.)

Contrary to our assumptions, dietary intake of aluminum, silicon, and titanium is not negligible when compared with potential intake of these elements from soil. The average adult dietary intakes of aluminum, silicon, and titanium are 15 mg/day, 7 mg/day, and .3 mg/day, respectively.⁸ The presence of these elements in food will lead to an overestimation of the amount of soil children eat.

CONCLUSIONS AND RECOMMENDATIONS

The estimates of soil ingestion generated in this report range over an order of magnitude. It is difficult to select the best measure of soil ingestion in toddlers on basis of the results, given the assumptions we had to make for this estimate. We need a better understanding of the metabolism of aluminum, silicon, and titanium in children. Studies of aluminum, silicon, and titanium levels in the feces of volunteers; studies of the excretion of these elements by hospitalized children; repetition of our study in an area with homogenous soil, and controlled studies of the methodology used are possible next steps. Careful attention to study methods and monitoring of dietary intake will undoubtedly lead to better estimates of soil ingestion. We hope that this study will stimulate further studies of soil ingestion by young children.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Table 1. Recovery of aluminum, silicon, and titanium from spiked pooled stool samples, analyzed by ICP.*

Element	Amount added (ug/g)	Number of samples	Mean amount recovered	S.D. (ug) (ug/g)	Percent recovered	
					Method A+	Method B#
Aluminum	0	3	575	52.1	--	--
	250	3	767	68.5	93	77
	500	5	1012	26.1	94	87
Silicon	0	3	2053	175.0	--	--
	750	3	2753	151.0	98	93
	1500	7	3075	125.0	87	63
Titanium	0	3	325	20.7	--	--
	229	3	509	31.0	92	80
	456	5	766	33.3	98	97

* Alternative methods of calculating recovery and their advantages and disadvantages have been discussed in the literature ^{15,16} Two methods have been used in this table.

+ Method A:

$$\% \text{ recovery} = \frac{\text{observed spiked conc.}}{\text{observed unspiked conc.} + \text{spike conc.}} \times 100.$$

Method B:

$$\% \text{ recovery} = \frac{\text{observed spiked conc.} - \text{observed unspiked conc.}}{\text{spike conc.}} \times 100.$$

ug/g = micrograms of element per gram of stool

Table 2. Age and sex distribution of study participants.

Age (years)	Sex		<u>Total</u>
	<u>Male</u>	<u>Female</u>	
1.0-1.49	24	11	35
1.5-1.99	7	7	14
2.0-2.49	9	5	14
2.5-3.0	2	0	2
Total	42	23	65

Table 3. Mean daily fecal mass for male and female toddlers.

<u>Sex</u>	<u>N</u>	Mean (<u>g/d</u>)	Median (<u>g/d</u>)	Geo. mean (<u>g/d</u>)	S.D. (<u>g</u>)	Range (<u>g/d</u>)
Male	42	7.5	7.1	6.9	3.0	1.9-15.7
Female	23	6.9	6.0	6.1	3.4	1.8-17.2
Total	65	7.3	6.7	6.6	3.2	1.8-17.2

g/d = grams per day

Table 4. Comparison of fecal aluminum, silicon, and titanium concentrations measured by XRF and ICP.

<u>Element</u>	Mean concentrations (mg/g)		Correlation Coefficient (R)
	<u>XRF</u>	<u>ICP</u>	
Aluminum	0.47	0.83	0.19
Silicon	3.24	3.65	0.92
Titanium	0.29	0.33	0.97

mg/g = milligrams of element per gram of stool

Table 5. Fecal aluminum, silicon, and titanium concentrations (mg/g) by ICP for the 65 children with stool ICP data.

<u>Element</u>	<u>Mean (mg/g)</u>	<u>Median (mg/g)</u>	<u>Geometric mean (mg/g)</u>	<u>Standard deviation (mg/g)</u>	<u>Range (mg/g)</u>
Aluminum	0.83	0.53	0.60	0.82	0.11- 5.10
Silicon	3.65	2.56	2.56	3.45	0.66-14.88
Titanium	0.34	0.12	0.08	0.54	<0.01- 2.83

mg/g = milligrams of element per gram stool

Table 6. Number of soil and dust samples available for the 65 children with stool ICP data.

		Soil Sample		
		<u>Present</u>	<u>Absent</u>	<u>Total</u>
Dust sample	Present	42	3	45
	Absent	17	3	20
	Total	59	6	65

Table 7. Aluminum, silicon, and titanium concentrations determined (by XRF) in composite front-back yard soil samples and dust vacuum cleaner-bag samples.

<u>Sample</u>	<u>Sample size</u>	<u>Mean (mg/g)</u>	<u>Median (mg/g)</u>	<u>Geometric mean (mg/g)</u>	<u>Standard deviation (mg/g)</u>	<u>Range (mg/g)</u>
Soil Aluminum	59	66.61	66.95	66.03	9.22	45.14-111.41
Soil Silicon	59	302.52	302.19	301.94	18.34	243.06-332.23
Soil Titanium	59	2.98	2.93	.42	2.95	2.38- 4.01
Dust Aluminum	45	33.65	34.19	29.91	13.77	5.37- 64.23
Dust Silicon	45	202.43	202.54	198.77	38.94	115.81-319.41
Dust Titanium	45	2.72	2.77	2.67	0.52	1.23- 3.84

mg/g = milligrams of element per gram of stool

Table 8. Estimated daily soil ingestion based on aluminum, silicon, and titanium for the 59 children with both stool ICP and yard soil data.

<u>Estimation method</u>	<u>Mean (mg/d)</u>	<u>Median (mg/d)</u>	<u>Geometric mean (mg/d)</u>	<u>Standard deviation (mg/d)</u>	<u>Range (mg/d)</u>	<u>95th per- centile (mg/d)</u>
Aluminum	181	121	128	203	25-1,324	584
Silicon	184	136	130	175	31- 799	578
Titanium	1,834	618	401	3,091	4-17,076	9,590

mg/d = milligrams of soil per day

Table 9. Estimated daily soil ingestion from the minimum of calculated ingestion based on aluminum, on silicon, and on titanium.

Element producing the lowest ingestion estimate	Number of children	% of children	Mean (mg/d)	Median (mg/d)	Geometric mean (mg/d)	S.D. (mg/d)	Range (mg/d)	95th percentile (mg/d)
Al	21*	36**	100	93	90	43	25-209	204
Si	24*	41**	159	112	110	168	37-708	675
Ti	14*	24**	34	14	16	36	4- 97	97
Total	59	100	108	88	65	121	4-708	386

*Number of children with the soil ingestion estimate for that element as the lowest of the estimates for aluminum, silicon, and titanium.

**Percentage of children with the soil ingestion estimate for that element as the lowest of the estimates for aluminum, silicon, and titanium.

mg/d = Milligrams of soil per day

Table 10. Estimated daily soil ingestion based on mean soil concentrations* of aluminum, silicon, and titanium for the 65 children with stool ICP data.

<u>Estimation method</u>	<u>Mean (mg/d)</u>	<u>Median (mg/d)</u>	<u>Geometric (mg/d)</u>	<u>S.D. (mg)</u>	<u>Range (mg/d)</u>
Aluminum	186	120	136	185	26-1,149
Silicon	181	127	127	171	33-738
Titanium	1,705	549	399	2,711	5-14,221

*See Table 7 for mean soil concentrations.

mg/g = milligrams of soil per day

Table 11. Estimated of daily dust ingestion (assuming no soil ingestion) for the 45 children with stool ICP and dust data.

<u>Estimation method</u>	<u>Mean (mg/d)</u>	<u>Median (mg/d)</u>	<u>Geometric mean (mg/d)</u>	<u>S.D. (mg/d)</u>	<u>Range (mg/d)</u>
Aluminum	515	289	326	634	33-3,474
Silicon	286	197	205	287	48-1,265
Titanium	2,068	871	510	3,006	5-13,923

mg/d = milligrams of dust per day

Table 12. Effect of various assumptions on estimated soil ingestion in this study.

Assumption	Effect on estimated soil ingestion if assumption not true
1. Removal of feces and urine from diapers is complete.	Decreased
2. Individual child's yard's composite soil concentrations reflect his or her ingested soil better than mean soil concentrations for area.	Increased or decreased
3. Dust ingestion is negligible (effect on sum of dust and soil ingestion).	Increased
4. Daily stool output averages 15 g per child.	Increased or decreased
5. Al, Si, and Ti are unabosrbed from the g.i. tract.	Increased
6. Dietary Al, Si, and Ti are negligible.	Decreased
7. Aluminum, silicon, and titanium were neither added to nor lost from the specimens during processing.	Increased or decreased

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